

# Kinase signalling pathways in endometriosis: potential targets for non-hormonal therapeutics

Brett D. McKinnon<sup>1,2,\*</sup>, Vida Kocbek<sup>1,2</sup>, Kostantinos Nirgianakis<sup>1,2</sup>,  
Nick A. Bersinger<sup>1,2</sup>, and Michael D. Mueller<sup>1,2</sup>

<sup>1</sup>Department of Obstetrics and Gynaecology, Inselspital, Berne University Hospital, Effingerstrasse 102, Berne CH-3010, Switzerland

<sup>2</sup>Department of Clinical Research, University of Berne, Murtenstrasse 35, Berne CH-3010, Switzerland

Correspondence address. E-mail: brett.mckinnon@dkf.unibe.ch

Submitted on July 31, 2015; resubmitted on November 10, 2015; accepted on December 8, 2015

## TABLE OF CONTENTS

- Introduction
  - Challenges of current endometriosis management
  - The endometriotic microenvironment
  - The extracellular influence on endometriotic cells
- Methods
  - The NF $\kappa$ B pathway in endometriosis
  - The MAPK pathways in endometriosis
  - The PI3K/AKT/mTOR pathway in endometriosis
- Conclusion
  - Limitations and challenges
  - Future directions

**BACKGROUND:** Endometriosis, the growth of endometrial tissue outside the uterine cavity, is associated with chronic pelvic pain, subfertility and an increased risk of ovarian cancer. Current treatments include the surgical removal of the lesions or the induction of a hypoestrogenic state. However, a reappearance of the lesion after surgery is common and a hypoestrogenic state is less than optimal for women of reproductive age. Additional approaches are required. Endometriosis lesions exist in a unique microenvironment characterized by increased concentrations of hormones, inflammation, oxidative stress and iron. This environment influences cell survival through the binding of membrane receptors and a subsequent cascading activation of intracellular kinases that stimulate a cellular response. Many of these kinase signalling pathways are constitutively activated in endometriosis. These pathways are being investigated as therapeutic targets in other diseases and thus may also represent a target for endometriosis treatment.

**METHODS:** To identify relevant English language studies published up to 2015 on kinase signalling pathways in endometriosis, we searched the Pubmed database using the following search terms in various combinations; 'endometriosis', 'inflammation', 'oxidative stress', 'iron', 'kinase', 'NF kappa', 'mTOR', 'MAPK' 'p38', 'JNK', 'ERK' 'estrogen' and 'progesterone'. Further citing references were identified using the Scopus database and finally current clinical trials were searched on the clinicaltrials.gov trial registry.

**RESULTS:** The current literature on intracellular kinases activated by the endometriotic environment can be summarized into three main pathways that could be targeted for treatments: the canonical IKK $\beta$ /NF $\kappa$ B pathway, the MAPK pathways (ERK 1/2, p38 and JNK) and the PI3K/AKT/mTOR pathway. A number of pharmaceutical compounds that target these pathways have been successfully trialled in *in vitro* and animal models of endometriosis, although they have not yet proceeded to clinical trials. The current generation of kinase inhibitors carry a potential for adverse side effects.

**CONCLUSIONS:** Kinase signalling pathways represent viable targets for endometriosis treatment. At present, however, further improvements in clinical efficacy and the profile of adverse effects are required before these compounds can be useful for long-term endometriosis treatment. A better understanding of the molecular activity of these kinases, including the specific extracellular compounds that lead to their activation in endometriotic cells specifically should facilitate their improvement and could potentially lead to new, non-hormonal treatments of endometriosis.

**Key words:** inflammation / signalling kinase / NF kappa B / mTOR / MAPK / microenvironment / treatment / drugs / endometriosis

## Introduction

Endometriosis is an estrogen-dependent condition characterized by the growth of endometrial epithelial and stromal cells outside the uterine cavity and is often accompanied by chronic pelvic pain, subfertility and an increased risk of ovarian cancer (Vercellini *et al.*, 2014). It is an extremely prevalent condition, occurring in 10% of women of reproductive age (Eskenazi and Warner, 1997) and up to 50% of women with infertility (Meuleman *et al.*, 2009) and represents a significant burden on the health care system (Simoens *et al.*, 2012). Although a number of theories have been proposed, the most widely accepted is the Sampson theory of transplantation where menstrual tissue, including viable endometrial epithelial and stromal cells, enter the peritoneal cavity via retrograde menstruation (Sampson, 1927). Once present, an innate or acquired characteristic of these endometrial cells and the inflammatory and hormonal microenvironment combine to facilitate lesion growth at multiple locations throughout the peritoneal cavity (Burney and Giudice, 2012).

Endometriosis is an extremely heterogeneous condition that was originally proposed to exist as three different entities: peritoneal endometriosis, ovarian endometrioma and adenomyotic nodules of the rectovaginal septum all of which develop through distinct pathogenic pathways (Nisolle and Donnez, 1997). More recent research, however, suggests that the different clinical presentations are actually a continuum of the same disease (Vercellini *et al.*, 2000) with shared origins (Somigliana *et al.*, 2004, 2007). Superficial peritoneal endometriotic lesions represent the least severe clinical presentation, followed by endometrioma and deeply infiltrating endometriosis (DIE), the most severe (Chapron *et al.*, 2009). DIE is defined by infiltration into the *muscularis propria* (Chapron *et al.*, 2010) and is further subcategorized by the invaded organ, which may be the bladder, uterosacral ligaments, intestines and/or vagina (Chapron *et al.*, 2003b). DIE lesions are most commonly associated with strong pain (Chapron *et al.*, 2003a) and represent the most complex clinical challenge (Abrão *et al.*, 2015).

## Challenges of current endometriosis management

The current European Society of Human Reproduction and Embryology (ESHRE) guidelines advocate endometriosis management via hormonal modulation with medical therapies, or the surgical removal of the lesions (Dunselman *et al.*, 2014). Both of these approaches, however, have significant shortcomings.

Hormonal modulation through medical therapies creates a hypoestrogenic environment with hormonal contraception, progestogens, anti-progestogens, gonadotrophin-releasing hormone analogues and aromatase inhibitors (Brown and Farquhar, 2014). This approach,

however, is inappropriate for patients with endometriosis-associated infertility who wish to conceive normally (Dunselman *et al.*, 2014). Furthermore, symptoms reoccur once treatment has ceased (Streuli *et al.*, 2013) and up to one-quarter of patients will have intolerable side effects, or not respond (Vercellini *et al.*, 2008). An inadequate response to medical therapies is believed to be a particular problem for DIE lesions (Vercellini *et al.*, 2009), possibly due to extensive fibrosis rendering them less susceptible to hormonal modulation (Remorgida *et al.*, 2005).

Surgical intervention is the primary treatment of choice for severe forms of endometriosis (Abrão *et al.*, 2015), such as symptomatic DIE that incorporate bowel or urethra stenosis, large adnexal masses or large endometrioma (Vercellini *et al.*, 2009; Meuleman *et al.*, 2011). A reduction in pelvic pain (Jacobson *et al.*, 2009), dyspareunia (Ferrero *et al.*, 2007) and an increase in fertility (Duffy *et al.*, 2014) is achieved via surgical intervention with a significant improvement in patient wellbeing and quality of life that can be extrapolated to significant savings for the health care system (Wullschleger *et al.*, 2015). Surgery, however, can be associated with complications, particularly in complex cases. Recurrence of the lesions (Shaw, 1992) and the painful symptoms (Duffy *et al.*, 2014) is also common.

Surgical removal of DIE lesions can be complex and outcomes are highly dependent on surgical skill. A recent meta-analysis revealed the complication rates for bowel resection anastomosis of DIE lesions have been measured as 2.7% of patients for rectovaginal fistulae, 1.5% for anastomotic leakage and 0.34% for pelvic abscesses. The less aggressive techniques had slightly lower rates, but were associated with an increase in recurrence from 5.8 to 17.6% (Meuleman *et al.*, 2011). The primary reason for recurrence is unclear but an incomplete resection of the lesion (Nirgianakis *et al.*, 2014) due to the complexity of the surgery or to the presence of occult endometriosis (Khan *et al.*, 2014) are possible.

## The endometriotic microenvironment

The peritoneal microenvironment is significantly altered in endometriotic women. Endometrial cells refluxed into the peritoneal cavity secrete chemokines (Lebovic *et al.*, 2001) creating a feed-forward loop (Hornung *et al.*, 2001) that stimulates the infiltration of immune cells (Halme *et al.*, 1983). Both endometriotic and immune cells (Laird *et al.*, 1993; Bersinger *et al.*, 2008, 2011) produce pro-inflammatory cytokines and prostaglandins (Badawy *et al.*, 1985; Wu *et al.*, 2005) and anti-inflammatory interleukins are suppressed (Santulli *et al.*, 2013) creating an inflammatory imbalance. Erythrocytes and menstrual debris enter the peritoneal cavity via retrograde menstruation resulting in increased iron concentrations (Arumugam and Yip, 1995; Iizuka *et al.*, 1998; Yamaguchi *et al.*, 2008) that accumulate in peritoneal macrophages (Lousse *et al.*, 2009) and mediate oxidative stress

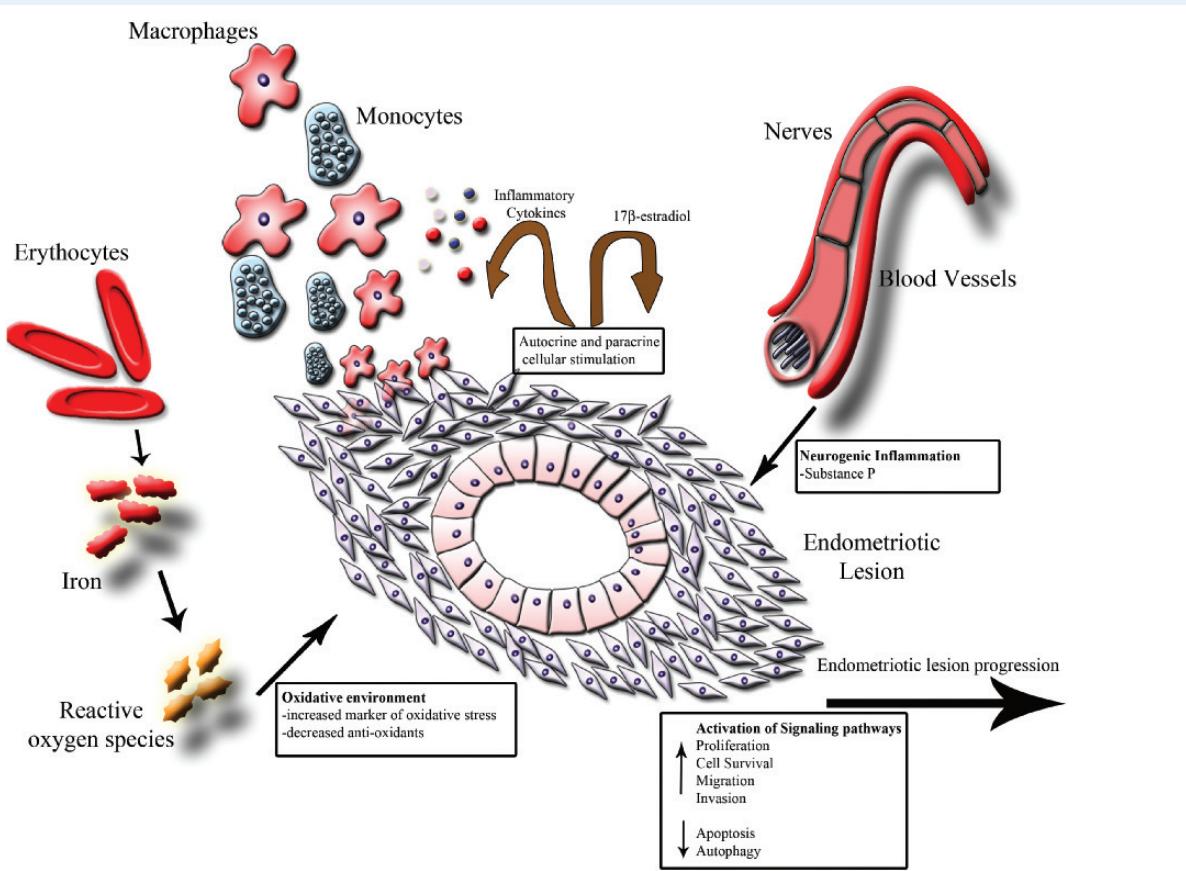
(Defrère *et al.*, 2008) in both the peritoneal fluid (Carvalho *et al.*, 2012) and the endometriotic cells (Murphy *et al.*, 1998; Oner-lyidoğan *et al.*, 2004; Ngô *et al.*, 2009; Seo *et al.*, 2010).

Once the lesions are established, local estrogen production begins through endometrial cell expression of aromatase p450 (Noble *et al.*, 1996) and a reduction in 17 $\beta$ -hydroxysteroid dehydrogenase type II (Zeitoun *et al.*, 1998). The overexpression of estrogen receptor (ER) $\beta$  in endometriotic stromal cells also alters their behaviour leading to a reduction in the expression of ER $\alpha$  (Xue *et al.*, 2007; Trukhacheva *et al.*, 2009) and possibly of progesterone receptors (PRs) (Bulun *et al.*, 2010). Finally, neuroangiogenesis leads to the infiltration of nerve fibres and blood vessels (Asante and Taylor, 2011) that supply nutrients and remove waste, as well as secreting neurogenic compounds (Sanfilippo *et al.*, 1992) that interact with endometriotic lesions (McKinnon *et al.*, 2013). These mechanisms create an altered endometriotic microenvironment characterized by an inflammatory imbalance, oxidative stress and increased iron concentrations that support the maintenance of the cells, while their continued growth is facilitated by estrogen production and neuroangiogenesis (Fig. 1).

## The extracellular influence on endometriotic cells

The ability of this altered microenvironment to support endometriotic cells is transmitted by kinase signalling pathways. In many diseases, the dysregulation of a protein kinase leads to unchecked cellular proliferation through stimulation of neoplastic processes resulting in a kinases-dependent tumour growth (Sawyers, 2003). Pharmaceuticals targeting these kinases is proving successful in the treatment of other tumours and is increasingly being examined as potential endometriosis treatments. Whether endometriosis exhibits kinase dependency is not yet clear, although inflammation (Lee and Hung, 2007), neurogenic mediators (Azzolina et al., 2003), steroid hormones (King et al., 2010) and both iron and oxidative stress (Alvarado-Díaz et al., 2015) interact with multiple kinase signalling pathways in endometriotic cells.

The interaction of the microenvironment and the endometriotic cells may also vary based on lesion subtype. DIE lesions have a significantly different microenvironment compared with lesions from other locations as they produce significantly more inflammatory cytokine mRNA (Bertschi *et al.*, 2013) and have higher peritoneal fluid IL-33 concentrations



**Figure 1** The endometriotic microenvironment. Endometriotic lesions exist in a unique microenvironment created by the interaction of multiple cells. Through retrograde menstruation epithelial and stromal endometrial cells, along with erythrocytes and other menstrual debris enter the peritoneal cavity. The endometrial cells attach to the underlying mesothelium and establish ectopic lesion that begin producing chemokines and hormones. These compounds can have both an autocrine and paracrine effect. Chemokines stimulate the infiltration of immune cells and hormones influence the endometriotic cells. Erythrocytes lead to increased iron concentrations, which in turn creates reactive oxygen species and an oxidative environment. The subsequent inflammatory, hormonal and oxidative environment leads to the stimulation of the signalling kinase pathways that facilitate endometriotic lesion progression.

(Santulli *et al.*, 2012) and oxidative stress markers than lesions from other locations (Santulli *et al.*, 2015a). Significantly higher concentrations of endometriosis-associated nerve fibres have also been observed in DIE lesions increasing the potential for neurogenic inflammation (McKinnon *et al.*, 2012b). Whether the extracellular environment of DIE lesions creates a specific influence is not clear, but the high concentrations of potential kinase stimulating components suggest DIE lesions may respond to kinase inhibition, as opposed to hormonal therapies.

All together this makes endometriosis a heterogeneous condition that poses a difficult clinical challenge, particularly for symptomatic DIE lesions. New therapeutic options are needed. Endometriotic lesions create a unique microenvironment capable of inducing kinase activity and potentially, a kinase-dependent lesion growth. Targeting these kinases may represent a potential novel treatment, and may also hold potential for DIE lesions. We therefore examined the relevant literature to identify published data on kinase activity in endometriotic tissue and to determine whether they were activated by components of the endometriotic extracellular environment. We focused on three specific pathways involving nuclear factor (NF)κB, mitogen-activated protein kinase (MAPK) or mammalian target of rapamycin (mTOR). We also assessed therapeutics that target these pathways and analysed their potential for future treatments.

## Methods

We identified relevant English language studies published up to 2015 via a search of the Pubmed database using the following search terms in various combinations: 'endometriosis', 'inflammation', 'oxidative stress', 'iron' 'kinase', 'NF kappa', 'mTOR', 'MAPK' 'p38', 'JNK', 'ERK' 'estrogen' and 'progesterone'. Further citing references were identified using the Scopus database and current clinical trials were identified using the clinicaltrials.gov trial registry.

## The NFκB pathway in endometriosis

NFκB is the nodal point of a primary inflammation stimulated signalling pathway that has a significant role in the immune response (Hayden *et al.*, 2006). The NFκB complex is assembled from two groups of proteins: the NFκB proteins, p105 and p100, which are truncated to p50 and p52, respectively, and the Rel proteins (c-Rel, REL B and p65). These proteins combine as either hetero or homodimeric complexes to form the NFκB complex of which the most common arrangement is the p50/p65 heterodimer (Ghosh *et al.*, 1998). Under resting state conditions, the dimeric NFκB/Rel complexes are bound to the inhibitor kappa beta protein (IκB). Binding between the NFκB and IκB keeps the complex sequestered to the cytosol (Fig. 2). Activation of cell surface receptors by the extracellular environment begins a cascading reaction that separates IκB and NFκB complex and allows for the translocation of NFκB to the nucleus and initiation of gene transcription. IκB removal from the NFκB complex is mediated by the IκB kinase (IKK) complex, which consists of two catalytic subunits IKK $\alpha$  and IKK $\beta$  and the regulatory subunit IKK $\gamma$  (Smale, 2011).

Two distinct cascading reactions, each controlled by the different catalytic subunits of the IKK complex, lead to NFκB activation. The canonical NFκB pathway is characterized by activity of the IKK $\beta$  catalytic subunit removing IκB from p65 and targeting it for ubiquitin ligase-mediated degradation (Ghosh and Karin, 2002). The alternative NFκB pathway is characterized by IKK $\alpha$  catalytic activity that is stimulated by NFκB inducing kinase (NIK). This catalytic subunit preferentially targets IκB proteins bound to the p100-Rel B dimers stimulating a partial proteasome degradation that creates the transcriptionally active p52-Rel B dimer (Oeckinghaus *et al.*,

2011). Both the canonical and alternative NFκB pathways lead to increased transcription of different genes and therefore mediate different immune functions (Bonizzi and Karin, 2004).

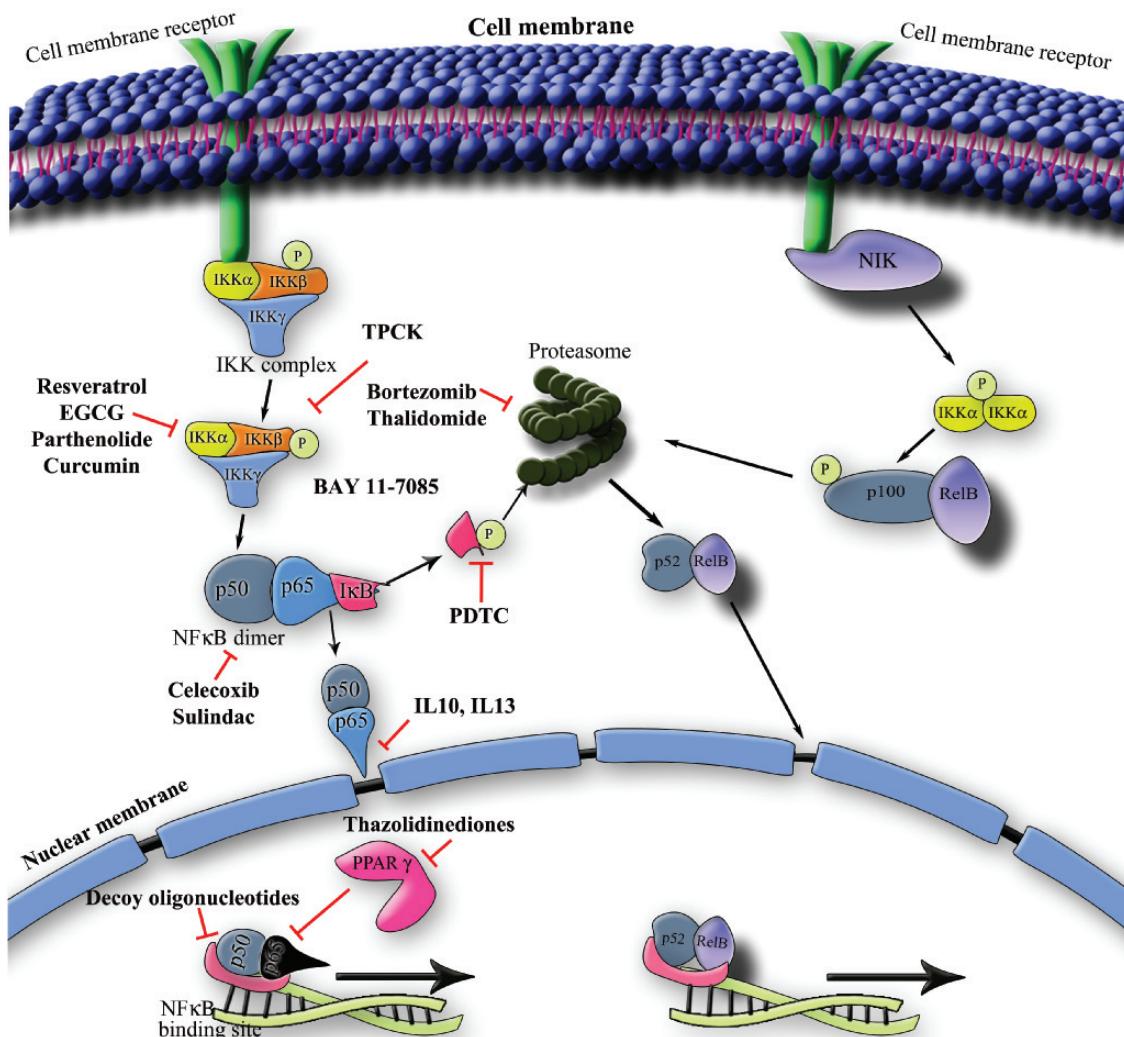
NFκB may represent a potential therapeutic target due to its constitutive activation in peritoneal endometriotic lesions (Gonzalez-Ramos *et al.*, 2007). An overexpression of NFκB has been confirmed in cultured endometriotic stromal cells (Sakamoto *et al.*, 2003) and peritoneal macrophages (Lousse *et al.*, 2008) isolated from women with endometriomas. Furthermore, in ovarian endometriomas, p65 expression has been correlated with recurrence (Shen *et al.*, 2008). *In vitro* evidence raises the possibility that the constitutive activation may be due to the endometriotic microenvironment. IL-1 $\beta$  stimulates NFκB with a subsequent increased production of inflammatory cytokines (Veillat *et al.*, 2009), including macrophage migration inhibitory factor (MIF) (Cao *et al.*, 2006) in endometrial stromal cells, as does tumour necrosis factor alpha (TNF $\alpha$ ) (Grund *et al.*, 2008) in the immortalized epithelial (12Z) cell line. In primary epithelial cells, 17 $\beta$ -estradiol stimulates NFκB nuclear translocation (Zhang *et al.*, 2010a) and progesterone withdrawal increases NFκB activity in the endometrium (King *et al.*, 2001). Interestingly, iron increases NFκB activity in endometriotic stromal cells (Alvarado-Díaz *et al.*, 2015) and it has been speculated that the alternative NFκB pathway may be responsible for the stimulation of inflammation by iron overload in endometriotic women (González-Ramos *et al.*, 2012). However, the contribution of iron to NFκB remains controversial (Hayakawa *et al.*, 2003).

There is also the significant possibility of an interaction between NFκB and peroxisome proliferator-activated receptor (PPAR) $\gamma$ , a nuclear transcription factor involved in the inflammatory response (Daynes and Jones, 2002) and implicated in the pain experienced by endometriotic women (Moravek *et al.*, 2009; McKinnon *et al.*, 2010). The exact mechanism by which PPAR $\gamma$  agonists attenuate the inflammatory response, however, is not yet clear, but previous evidence has shown that the natural ligand for PPAR $\gamma$ , 15-deoxy-delta-12, 14-prostaglandin J2 (15dPGJ2) also represses NFκB (Castrillo *et al.*, 2000; Straus *et al.*, 2000), raising the possibility that some of the anti-inflammatory effects ascribed to the PPAR $\gamma$  agonist may be PPAR $\gamma$  independent. In endometrial stromal cells, both pioglitazone and ciglitazone attenuate the production of IL-6 and IL-8 in a PPAR $\gamma$ -independent mechanism (McKinnon *et al.*, 2012a) and pioglitazone significantly reduces the concentration of TNF $\alpha$ -stimulated p65 (Ohama *et al.*, 2008).

### Targeting the NFκB pathway in endometriosis

As NFκB regulates numerous physiological processes and contributes to the pathology of several human diseases, there has been a great deal of interest in designing pharmacological methods to intervene in its activity (Gilmore and Herscovitch, 2006). Given the huge number of compounds already developed, we have focused only on those that have shown either *in vitro* or clinical potential in endometriosis and divided these into molecules that function prior to the removal of IκB from the NFκB complex (upstream) and those that function after the removal of IκB and the translocation of the complex to the nucleus (downstream).

Upstream modulation of NFκB activity has been trialled in endometriosis via inhibition of the catalytic subunits that mediate IκB phosphorylation and its removal from the NFκB complex and subsequent proteasomal degradation (Fig. 2). BAY 11-7085, a synthetic compound that inhibits IκB phosphorylation (Pierce *et al.*, 1997), decreased cell proliferation and DNA synthesis and induced apoptosis in endometriotic stromal cells (Nasu *et al.*, 2007). In a heterologous nude mouse model, it decreased lesion size and increased apoptotic markers (González-Ramos *et al.*, 2008). Bortezomib, a proteasome inhibitor, reduced the endometriotic lesion size in a transplanted endometriosis model using Wistar rats and decreased proliferating cell nuclear antigen (PCNA) and Ki67 expression (Celik *et al.*, 2008), whereas *N*-Tosyl-L-Phenylalanine Chloromethyl ketone (TPCK) also showed anti-NFκB activity in primary stromal cells isolated from endometrioma (Yamauchi *et al.*, 2004). Pyrrolidine dithiocarbamate (PDTC),



**Figure 2** The NF-κB signalling pathway and its inhibition in endometriosis. Binding to cell membrane receptors stimulates both the canonical and alternative NF-κB signalling pathways. Stimulation of the canonical NF-κB pathway leads to the phosphorylation of IKKβ. IKKβ is part of the IKK complex along with IKKα and IKKγ and an activated IKKβ phosphorylates the inhibitory protein IκB preferentially on p50–p65, removing it from the complex and targeting it for proteasomal degradation. The unbound p50–p65 complex translocates into the nucleus and stimulates gene transcription. The PPARγ nuclear transcription factor may also interact with p50–p65 complex and suppress gene transcription. Extracellular molecules, including inflammatory mediators, oxidative stress markers and iron also activate the alternative NF-κB pathway. Binding of these molecules to cell membrane receptors leads to activation of the NIK, which in turn phosphorylates IKKα dimers preferentially that remove IκB from the p100–Rel B complex. Removal of the IκB protein allows a partial degradation of the p100 protein to p52 and the subsequent p52–Rel B dimer to translocate to the nucleus and stimulate gene transcription.

which functions as both an antioxidant effects and IκB-ubiquitin ligase (Hayakawa et al., 2003) decreased inflammation, angiogenic factors and matrix metalloproteinases (MMP) *in vitro* in both endometrial epithelial (Zhang et al., 2011) and stromal cells (Zhang et al., 2010a, b), all of which were preferential in endometriotic compared with endometrial cells. Furthermore, in a heterologous transplanted endometriosis model in Wistar rats, PDTC mediated a reduction in lesion size (Celik et al., 2008).

Downstream of the NF-κB complex, it is also possible to inhibit the transcriptional activity of this pathway via disruption of NF-κB translocation to the nucleus and the subsequent DNA binding (Fig. 2). The anti-inflammatory cytokines IL-10 and IL-13 suppress nuclear localization of NF-κB and increase the IκB mRNA transcription (Lentsch et al., 1997) and in endometriotic stromal cells, IL-10 treatment significantly reduces the production of

TNFα-induced IL-6 but not IL-8 production (Tagashira et al., 2009). Blocking the specific NF-κB DNA-binding sites at promoter regions with decoy oligonucleotides is another possible strategy (Khaled et al., 1998) that has been used successfully with endometriotic stromal cells *in vitro* as it was shown to suppress IL-1β-induced RANTES production and MCP-1 activity (Xiu-li et al., 2009).

Pharmaceuticals with off-target effects on NF-κB have also been considered for endometriosis treatment. Thalidomide inhibits NF-κB through the suppression of IκB degradation (Majumdar et al., 2002). Treatment of endometriotic stromal cells with thalidomide inhibited TNFα-stimulated IL-8 production and secretion (Yagyu et al., 2005) and reduced the size of autologous transplanted endometriotic lesions in rat models (Azimrad et al., 2014). Thiazolidinediones, ligands for PPARγ, which may have PPARγ-independent

mechanism in endometriotic stromal cells (McKinnon *et al.*, 2012a) and originally developed for diabetes treatment, reduced the size of endometriotic lesions in both rats (Lebovic *et al.*, 2004) and primates (Lebovic *et al.*, 2007). These drugs, however, also produce adverse effects on skeletal health (Bodmer *et al.*, 2009). Non-steroidal anti-inflammatory drugs (NSAIDs), such as celecoxib, inhibit cyclooxygenase (COX)-2 and also interact with NF $\kappa$ B in leiomyoma cells (Park *et al.*, 2014). In an *in vitro* experiment celecoxib also decreased cellular proliferation of endometrial epithelial cells (Olivares *et al.*, 2008). Sulindac also decreased RANTES through an NF $\kappa$ B mechanism (Wieser *et al.*, 2005). However, neither of these NSAIDs reduced the size of a surgically induced endometriotic lesion in a mouse model significantly more than any other NSAIDs (Efstathiou *et al.*, 2005).

Natural occurring compounds may also represent possible endometriosis treatments, mediated through their antioxidant effects on NF $\kappa$ B. Resveratrol, a compound present in red wine, modulates NF $\kappa$ B activity (Leiro *et al.*, 2005) and significantly reduced the size of surgically induced endometriotic lesions of nude mice (Bruner-Tran *et al.*, 2011) and reduced vascular density in a BALB/c mouse model (Ricci *et al.*, 2013). In both *in vitro* and animal models resveratrol reduced cell proliferation and increased apoptosis of endometrial epithelial cells (Ricci *et al.*, 2013; Ruzditzis-Auth *et al.*, 2013) as well as reducing peritoneal fluid MCP1, VEGF (Ergenoglu *et al.*, 2013; Ozcan Cenksoy *et al.*, 2015), IL-6, IL-8 and TNF $\alpha$  concentrations (Bayoglu Tekin *et al.*, 2015). Similarly, epigallocatechin-3-gallate (EGCG) a catechin found in green tea also interacts with NF $\kappa$ B (Khan *et al.*, 2006) and significantly reduced surgically induced endometriotic lesions in mice (Ricci *et al.*, 2013). Parthenolide, the active ingredient from the medical herb feverfew (*Tanacetum parthenium* L.), inhibited NF $\kappa$ B activity (Kwok *et al.*, 2001) and reduced the inflammatory response in endometriotic stromal cells isolated from endometriomas (Takai *et al.*, 2013). Curcumin, a naturally occurring polyphenol (Cao *et al.*, 2005), attenuated IL-1 $\beta$  induced MIF secretion (Veillat *et al.*, 2009) and TNF $\alpha$ -induced inflammation (Kim *et al.*, 2012) in endometriotic stromal cells, as well as reducing MMP3 expression and lesion size in BALB/c mice (Jana *et al.*, 2012).

It is possible that these compounds mediate anti-endometriotic activity. Owing to their low concentrations in the commonly consumed products of which they are found, it is unlikely, however, that they will produce lasting effects through natural consumption. However, through a manufacturing process it may be possible that the concentrations used to produce the effects observed *in vitro* and in animal models can be reproduced. Whether they will be at concentrations that are also clinically effective in human studies is not yet clear as significantly more information is still required about their bioavailability, metabolism and potential side effects.

There is also the potential for combinational therapy that incorporates an NF $\kappa$ B targeting compound with another molecule. The combination of celecoxib with the PPAR $\gamma$  agonist rosiglitazone significantly reduced the size of surgically induced lesions in mice (Olivares *et al.*, 2011) compared with the individual use of these drugs, although an antagonizing effect was observed when pairing celecoxib with the aromatase inhibitor anastrozole (Olivares *et al.*, 2013). Pycnogenol, from the bark of the French maritime pine (*Pinus pinaster*), has shown anti-NF $\kappa$ B activity in endothelial cells (Peng *et al.*, 2000) and used in combination with oral contraceptives showed promising results on dysmenorrhea, compared with use with contraception alone (Maia *et al.*, 2013, 2014). Resveratrol and oral contraceptives in combination also showed a greater decrease in aromatase and COX expression than in individual therapy (Maia *et al.*, 2012).

The adverse effects associated with modulating such a ubiquitously employed pathway may also limit therapeutic targeting of the NF $\kappa$ B pathway. Given the importance of NF $\kappa$ B to immune regulation, it may not be feasible to inhibit this pathway long-term as it may suppresses the host-immune response and leave the patient vulnerable for infection, an effect observed in animal models (Lavon *et al.*, 2000). Furthermore, a number of the targeted mechanisms in this pathway are regulatory proteins

that control numerous other functions within the cell and thus their inhibition may also lead to other unwanted side effects (Yamamoto and Gaynor, 2001).

Both embryotoxicity and teratogenicity are also important considerations given the demographic characteristics of endometriotic women. Of the drugs that interact with the NF $\kappa$ B pathway, thalidomide has a dire history and will be unlikely to have a useable reputation for women with endometriosis. PDTC has also shown some teratogenicity on zebrafish models (Tilton *et al.*, 2006) and the thiazolidinediones are categories C class pregnancy drugs and are currently not indicated during pregnancy. Sulindac also produced cleft palates in mouse models (Montenegro and Palomino, 1990) and high concentration of resveratrol was toxic in chick embryo toxicity assays (Venturelli *et al.*, 2013). Lastly, the parthenolide like compounds have recently been indicated as possibly embryotoxic (Amorim *et al.*, 2013).

### Summary

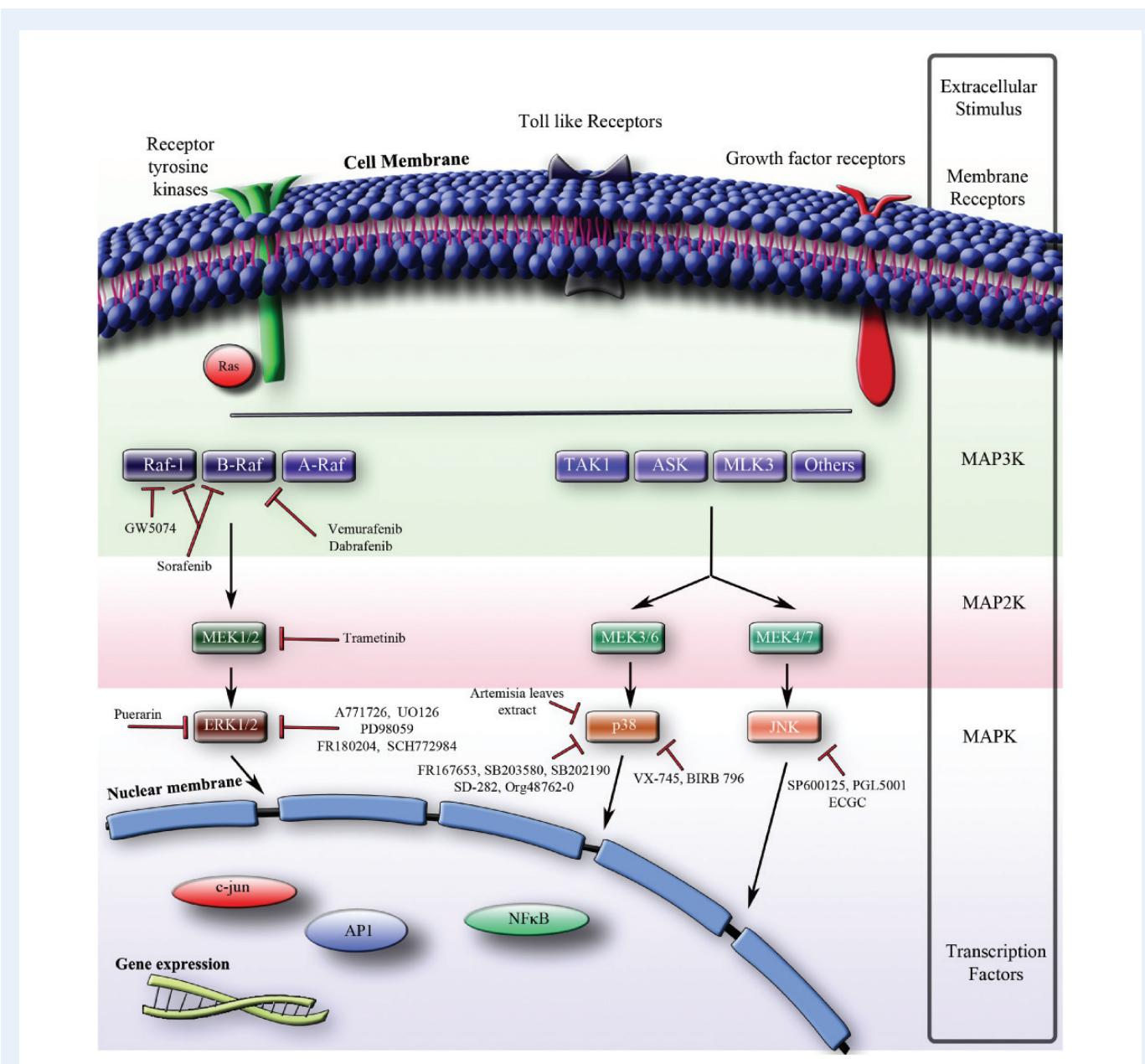
A constitutive activity of NF $\kappa$ B has been observed in endometriotic cells both *in vivo* and *in vitro*. Furthermore, inflammation, oxidative stress and hormones stimulate this pathway in endometriotic tissue and it therefore represents a potential target for endometriosis treatment. Given the central role of NF $\kappa$ B in mediating the immune response however, it is a concern that targeting its activity might also impair the body's natural ability to remove ectopic tissue. Targeting this pathway successfully therefore requires a balance between the suppression of the immune response and the induction of its apoptotic activity. Both upstream and downstream modulation of NF $\kappa$ B are viable approaches with particular promise in targeting proteasomal degradation of I $\kappa$ B. Such a balance may be achievable by combining a moderate inhibition of NF $\kappa$ B through naturally occurring compounds with additional targets, similar to other drugs that have off-target effects on this pathway. However, problems with reputation (thalidomide) and adverse side effects (thiazolidinediones) of some compounds will most likely limit their clinical applications. The ability of naturally occurring compounds to inhibit NF $\kappa$ B and their minimal side effects may provide the opportunity to combine these compounds with other drugs.

## The MAPK pathways in endometriosis

The MAPK pathways encompasses a collection of kinase signalling pathways, organized in a three tier hierarchical structure (1st-MAPK, 2nd-MAP2K and 3rd-MAP3K) with abundant crosstalk, that play a significant role in linking the extracellular environment with fundamental cellular responses. The MAPK signalling kinases are subdivided into the three families (Fig. 3): extracellular signal-regulated kinase (ERK), p38 and c-Jun-N terminal kinase (JNK) (Yoshino *et al.*, 2004). Within these subfamilies, six distinct terminal MAPKs have been characterized; ERK1/2, ERK3/4, ERK5, ERK7/8, which comprise the ERK family, JNK1/2/3, which make up the JNK family, and the p38 subunits  $\alpha$ / $\beta$ / $\gamma$ / $\delta$ , which comprise the p38 family (Dhillon *et al.*, 2007). The extracellular environment activates all three pathways with ERK predominantly activated by inflammation and growth factors and JNK and p38 predominantly activated by stress and inflammation. Once activated, the MAPKs initiate a cellular response via nuclear transcription factors.

### The ERK1/2 pathway

The ERK pathway is the most comprehensively studied of the mammalian MAPK pathways and was once synonymous with cell proliferation, although is now known to regulate other cellular responses (Dhillon *et al.*, 2007). At the cell membrane receptor, tyrosine kinases associate with small guanosine triphosphate proteins (GTPases) known as Ras (H, K and N-Ras). Once activated, these Ras GTPase mediate the tertiary Raf kinases, which in turn activates the secondary kinases MEK1/2 and subsequently the terminal kinase ERK1/2 (Little *et al.*, 2013; Fig. 3). The downstream effects of ERK pathway activation is the regulation of over 160 proteins, most of which are nuclear and alter gene expression (Yoon and Seger, 2006).



**Figure 3** MAPK pathways and their inhibition in endometriosis. The MAPK pathways is a collection of signalling pathways organized in a three tier structure. Through a series of membrane receptors, including cytokine receptors, toll-like receptors and growth factor receptors, the MAPK pathways are stimulated by many components of the endometriotic microenvironment. These membrane receptors stimulate a series of MAP3K signalling molecules that transmit this signal to the secondary MAP2K kinases, followed by the MAPK kinases. The ERK1/2 pathway is predominantly activated upstream by the Raf kinases (Raf-1, B-Raf and A-Raf), which have become a significant target for pharmaceutical modulation. These kinases signal through MEK1/2 to activate ERK and initiate nuclear translocation. The p38 and JNK pathways share a number of common upstream molecules in the MAP3K level that include TAK1, ASK, MLK3 level but diverge at the secondary MAP2K level with MEK3/6 mediating p38 activation and MEK4/7 mediating JNK activation. Once activated all three MAPK translocate into the nucleus and bind to transcription factors. These pathways can be targeted at numerous levels and the pharmaceutical compounds that have been trialled in endometriosis are marked at their location of action.

The increased ERK activation in endometriotic tissue suggests that it may have a role in endometriosis pathogenesis. Increased phosphorylated ERK has been reported in primary eutopic epithelial cells (Yotova et al., 2011; Matsuzaki and Darcha, 2015), as has a prolonged phosphorylation of ERK in endometrial stromal cells from women with endometriosis compared with women without endometriosis (Velarde et al., 2009). Furthermore, in both epithelial and stromal cells *in vitro* there is a significantly increased

phosphorylation of ERK in cells derived from endometriomas (Ngô et al., 2010) and DIE (Leconte et al., 2011) than in cells derived from normal endometrium. The factors that lead to a constitutive activation of ERK in endometriosis are not yet resolved, although one possibility that presents an attractive hypothesis is the reduction in the inactivating enzyme dual-specificity phosphatase (DUSP2) (Wu et al., 2011), through a hypoxia induced expression of miRNA-20a in endometriotic tissue (Lin et al., 2012).

The endometriotic microenvironment may stimulate increased ERK activity in ectopic cells. Both TNF $\alpha$  and IL-1 $\beta$  activate ERK and induce the expression of IL-8 and IL-6, although only IL-1 $\beta$  induced IL-8 secretion and COX2 production could be attenuated by the ERK1/2-specific inhibitor PD98059 (Yoshino *et al.*, 2004). Another study, however, found that ERK inhibition had no effect on the IL-1 $\beta$ -mediated COX2 expression in endometriotic stromal cells, but that it was rather through p38 activation (Huang *et al.*, 2013). TGF $\beta$ -induced ERK activation through a Raf-dependent pathway has also been identified in endometrial epithelial and stromal cells (De La Garza *et al.*, 2012). The chemokine MCP1 also elicits a significant induction of PGE2 (Carli *et al.*, 2009) as well as VEGF, IL-8 and MCP-1 via an ERK-specific pathway in human endometriotic cells (Veillat *et al.*, 2010), and PGE2 in turn activates ERK in ectopic endometrial stromal cells (Sun *et al.*, 2003).

Oxidative stress may also contribute to ERK activation. H<sub>2</sub>O<sub>2</sub> induces ERK phosphorylation in endometriotic stromal cells (Yoshino *et al.*, 2004) with a stronger induction compared with stromal cells from women without endometriosis (Andrade *et al.*, 2013). An increase in oxidative stress markers was observed in stromal and epithelial cells derived from women with endometriosis in a similar pattern to phosphorylated ERK levels, however, no direct relationship between oxidative stress and pERK activation was confirmed. Endocrine disruptors, such as diethylhexyl phthalate (DEHP) have also been linked with a possible pathogenesis of endometriosis through the induction of oxidative stress and stimulation of ERK activity (Cho *et al.*, 2015).

Estrogen also regulates ERK activation in endometriosis. Treatment with 17 $\beta$ -estradiol increases phosphorylated ERK expression in eutopic epithelial cells from women with and without endometriosis at similar rates between all cell types (Zhang *et al.*, 2010a). Treatment of ESC with E2 conjugated to bovine serum albumin (E2-BS) also increases phosphorylated ERK expression in a dose-dependent manner (Cheng *et al.*, 2012), indicating the effects are mediated at the cell membrane, as E2-BS cannot penetrate cells. This effect may also occur on immune cells with 17 $\beta$ -estradiol stimulating the release of MCP1 through activation of ERK in monocytes isolated from an endometriotic pelvic cavity (Lee *et al.*, 2012). Furthermore, in endometrial epithelial cells, TNF $\alpha$ -induced activation of ERs mediates an increase in ERK activation (Gori *et al.*, 2011).

#### The p38 pathway

Environmental stress stimuli including heat, osmotic shock and inflammatory cytokines influence the p38 MAPK pathway (Zarubin and Han, 2005). This diverse range of stimuli is indicative of the numerous tertiary level (MAP3K) kinases that participate in p38 activation (Fig. 3). These tertiary kinases include, but are not limited to TAK1 (Taniguchi *et al.*, 2009), ASK1, DLK/MUK/ZPK (Zarubin and Han, 2005). Many MAP3Ks stimulate both p38 and JNK, resulting in a convergence of the two pathways. Divergence of these two pathways occurs at the secondary kinase level with the activation of MEK3 and MEK6 kinases leading to the phosphorylation of p38 at a conserved amino acid sequence, threonine–glycine–tyrosine. Four isoforms of p38 have been characterized:  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ , of which p38 $\alpha$  is the best characterized. Upon activation p38 $\alpha$  translocates into the nucleus and activates nuclear transcription factors (Fig. 3).

At present, there is little data to confirm an over-activation of p38 in endometriotic cells. The endometriotic microenvironment, however, contains high concentrations of numerous molecules that activate this pathway, suggesting that constitutive activation in ectopic endometrial cells is possible. It has been suggested that in normal endometrium p38 activity is stronger in epithelial than in stromal cells (Seval *et al.*, 2006), although most of the current data has been collected in stromal cells. In endometriotic stromal cells, IL-1 $\beta$ , TNF $\alpha$  and H<sub>2</sub>O<sub>2</sub> stimulate p38 phosphorylation, while its suppression attenuates IL-1 $\beta$ -induced IL-6, IL-8 (Yoshino *et al.*, 2004) and VEGF secretion (Huang *et al.*, 2013), as well as COX2 mRNA production (Yoshino *et al.*, 2004). MIF induces VEGF, IL-8 and MCP-1 secretion

through p38 activation (Veillat *et al.*, 2010), as well as reduced COX2 expression, which may be specific to p38 (Carli *et al.*, 2009). In the immortalized *in vitro* epithelial model of peritoneal endometriotic cells (12Z), TNF $\alpha$  induces activation of p38 and concurrent treatment with specific inhibitors blocks IL-8, IL-6, MCP-1 and granulocyte macrophage colony-stimulating factor (GMCSF) secretion, as well as N-cadherin mRNA production (Grund *et al.*, 2008).

The activation of p38 may have a significant role in the regulation of non-endometriotic cells in the peritoneal microenvironment. MCP1 release from monocytes after treatments with peritoneal fluid is attenuated by a specific p38 inhibitor (Lee *et al.*, 2012), although this occurs equally in cells from women with and without endometriosis. IL-1 $\beta$  stimulates the thymic stromal lymphopoietin expression in Th2 cells by p38 inhibitors (Urata *et al.*, 2012). CCL20-induced Th17 cell recruitment to the peritoneal cavity of endometriotic women is regulated by p38 and other MAPK pathways (Hirata *et al.*, 2010). In a feed-forward mechanism, the Th17 cells in turn secrete IL-17 which induces IL-8 secretion through p38 and other MAP kinases pathways in endometriotic stromal cells (Hirata *et al.*, 2008). Lastly, p38 activation occurs in sensory nerve cells of the rostral–ventromedulla in a BALB/c mouse with surgically induced endometriosis (Chen *et al.*, 2015), suggesting a possible role for this pathway in inflammation-mediated endometriotic pain (McKinnon *et al.*, 2015).

Estrogen may also regulate p38 in endometriosis. Estradiol treatments of endometrial stromal cells increase p38 phosphorylation within two minutes and can be inhibited by ER antagonists (Seval *et al.*, 2006). 17 $\beta$ -estradiol stimulates p38 activation via ER $\beta$  in endometrial stromal cells (Chen *et al.*, 2014) and, in combination with the endocrine disruptor 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), influences macrophage polarization into an M2 phenotype, which is reduced by p38 inhibition, but not via JNK or ERK inhibition (Wang *et al.*, 2015). Another endocrine disruptor diethylhexyl phthalate increases the generation of ROS and decreases anti-oxidant enzymes through both ERK and p38 (Cho *et al.*, 2015), indicating the possible influence of environmental factors on this pathway.

Given the complexity of the microenvironment, it is not surprising that negative feedback loops exist to limit the influence of chronic inflammation and the p38 pathway may have a significant role in this negative regulation. Lipoxin A4 (LXA4) activates biochemical pathways necessary for the resolution of acute inflammation (Serhan *et al.*, 2008). LXA4 attenuates inflammation, angiogenic markers and estrogen metabolism as well as the endometriotic lesion itself in a surgically induced C57BL/6J mouse model (Kumar *et al.*, 2014) and importantly this effect of LXA4 is mediated through the p38 pathway in endometriotic stromal cells (Wu *et al.*, 2014). Sheddases also function in a feedback mechanism by cleaving receptors from the cell membrane. Sheddases activate MAPK pathways inducing A disintegrin and Metalloproteinases (ADAM)-10 and 17 that influence the receptor and ligand composition at the membrane, resulting in constitutive action of compensatory pathways, including p38 (Miller *et al.*, 2013).

#### The JNK pathway

Environmental stimuli for the JNK pathway include cytokines, growth factor deprivation, and G protein coupled receptors and stress signalling (Weston and Davis, 2002). In this pathway, the JNK protein represent the terminal (MAPK) kinase with up to 10 isoforms of JNK identified through alternative splicing of three different genes (*jnk1*, *jnk2* and *jnk3*). JNK can be activated upstream via the MEK4 and MEK7 kinases, which in turn are activated by several MAP3Ks that share similarities with those of p38, including TAK1 (Taniguchi *et al.*, 2013), MEKK1-4, MLL2/3, YTpL-2, DLK, TAO1/2 (Dhillon *et al.*, 2007). Stress signalling pathways feature a large number of MAP3K, reflecting the many possible molecules that can mediate a stress response. Once activated, the terminal kinase JNK translocates to the nucleus and activates transcription factors, of which c-Jun is a major target, enhancing AP-1 transcriptional activity (Adler *et al.*, 1992). JNK and NF $\kappa$ B often operate

in opposition, as anti-apoptotic effects of TNF $\alpha$  stimulation are mediated by NF $\kappa$ B-induced genes that suppress JNK activity (Javelaud and Besançon, 2001; Tang et al., 2002; Fig. 3).

Similar to p38, there is currently little data on whether there is an over-activation of the JNK pathway in endometriotic cells. Additionally, p38 and JNK share many activating molecules and upstream regulators. IL-17 (Hirota et al., 2005), IL-4 (OuYang et al., 2008) and IL-1 $\beta$  (Urata et al., 2012) mediate JNK phosphorylation, as does IL-1 $\beta$ , TNF $\alpha$  and H<sub>2</sub>O<sub>2</sub> (Yoshino et al., 2004). One extracellular molecular that may be specific to JNK activation is indoleamine 2,3-dioxygenase-1 (IDO1), as treatment of endometrial stromal cells with this compound stimulates a phosphorylation of JNK, but not of ERK or p38, and is able to stimulate an increase in proliferation, p53 expression and COX2 and MMP9 production (Mei et al., 2013). Estrogen may also play a role, as stimulation of TSLP by estrogen induces JNK phosphorylation and the subsequent secretion of IL-8 and MCP-1 (Chang et al., 2014). It has also been confirmed from miRNA profiling that some miRNAs in endometriotic tissue interact with downstream targets of the JNK pathway, such as c-jun (Teague et al., 2010).

#### Targeting the MAPK pathways in endometriosis

Given the upstream convergence of the three MAPK pathways (ERK1/2, p38 and JNK) attempts have been made to target shared upstream mediators. Specific inhibitors of B-raf, vemurafenib and dabrafenib, have been approved for use in melanoma, however, significant side effects, including the development of cutaneous squamous-cell carcinomas, exist (Su et al., 2012). Similar side effects have also been observed for the MEK inhibitor trametinib (Menzies et al., 2015), although at a lower frequency than for dabrafenib. Raf-1 represents another upstream mediator of ERK activity and inhibition with GW5074 attenuated EM42 and primary stromal cells proliferation and invasion (De La Garza et al., 2012).

Sorafenib is a multi-kinase inhibitor which has activity on the MAPK pathway at both Raf-1 and B-RAF and also has activity on receptor tyrosine kinases VEGF receptor 1, 2 and 3, platelet-derived growth factor receptor  $\beta$  (PDGFR- $\beta$ ) and c-Kit (Wilhelm et al., 2004). A significant decrease in endometrial stromal cell proliferation, as well as a reduction in surgically induced endometriotic lesions in a heterologous nude mouse model, was observed with high concentration treatments of sorafenib (Leconte et al., 2015). Sorafenib has also been associated with numerous side effects, the most common of which include palmoplantar erythrodysesthesia which occurs in 76.3% of patients, diarrhoea (68.8%), alopecia (67.1%), rash (50.2%), fatigue (49.8%), weight loss (46.9%), hypertension (40.6%) and anorexia (31.9%) (Krajewska et al., 2015). In a phase III clinical trial on thyroid cancer patients, these side effects lead to dose interruptions, reductions and withdrawals in 66.2, 64.3 and 18.8% of patients, respectively, over a 28 day treatment cycle (Brose et al., 2014).

Additional teratogenic and embryogenic effects should also be considered with the MAPK targeting drugs. Vemurafenib can cross the placenta in rat models, although no teratogenic effects were observed (Grunewald and Jank, 2015). In humans, its use during pregnancy was documented in one patient who experienced fetal growth retardation during gestation with a subsequent recovery after birth (Maleka et al., 2013). Dabrafenib on the other hand has shown reproductive toxicity in rats and dogs (Grunewald and Jank, 2015). Data from clinical trials on reproduction, however, are limited due to ethical concerns and while animal studies have been performed, the significant variations between the reproductive systems of different animals make it difficult to draw effective conclusions from these studies.

It is possible that a reduced side effects profile may be achievable if further downstream targets with an over-activity in endometriotic cells are identified. At the tertiary kinase level several small molecular weight inhibitors have been specifically designed to target ERK, p38 or JNK. The inhibition of ERK in endometriosis-derived cells with A771726 (Leconte et al., 2011), UO126 (Matsuzaki and Darcha, 2015) and higher concentrations of

PD98059 (Ngô et al., 2010) decreased cell proliferation. Some of these have reached the stage of animal and clinical trials for other chronic inflammatory conditions and may be worth investigating in endometriosis. FR180204 alleviates clinical arthritis and hypersensitivity elicited by an inflammatory reaction in collagen induced arthritis in a DBA/1 mouse model (Ohori et al., 2007) and SCH772984 has been successful in preclinical testing in cell lines that were BRAF and MEK inhibitor-resistant (Morris et al., 2013).

Small molecular weight inhibitors have also been developed for p38 and trialled both *in vitro* and in animal studies for use in endometriosis. The subcutaneous injection of 30 mg/kg FR167653 mediated a reduction in endometriotic lesion size and reduced both IL-6 and MCP-1 in the peritoneal fluid of BALB/c mice after a surgical transplantation of endometriotic lesions (Yoshino et al., 2006). SB203580 reduced IL-1 $\beta$  secretion and endometriotic lesion size in endometriotic stromal cells (Huang et al., 2013), as well as reducing TNF $\alpha$ , IL-1 $\beta$ , MMP3 and MMP9 mRNA and protein concentrations in cells isolated from the peritoneal cavity of an induced mouse model of endometriosis (Zhou et al., 2010). SB202190 attenuated cell proliferation of endometriotic stromal cells (OuYang et al., 2008). However, p38 $\alpha$  inhibitors are plagued by liver toxicity that suggests specific on-target effects (Xu et al., 2008) that may significantly limit their potential use. Both VX-745 and BIRB 796 failed phase II clinical trials due to high liver toxicity (Dambach, 2005). The inhibition of p38 $\alpha$  may also antagonize the JNK-c-jun pathway, as judged by a conditional deletion in mice (Hui et al., 2007).

The utility of targeting JNK in endometriosis is yet to be fully realized, as it is the least characterized pathway. SP600125 is a small molecular weight inhibitor developed to specifically target JNK (Bennett et al., 2001) and initial studies in both mouse models and in *in vitro* analysis of human synoviocytes, as a model of rheumatoid arthritis, it was capable of reducing the inflammatory response (Han et al., 2001). SP600125 also attenuated IL-1 $\beta$  induced inflammation in endometriotic stromal cells (Yoshino et al., 2004). The bentamapimod, PGL5001 is registered for a Phase IIa clinical trial in the treatment of endometriosis although there is very little publicly available information on the effectiveness of this compound *in vitro* (clinicaltrials.gov registry number; NCT01630252). However, similar to p38 inhibitors, it is possible that JNK inhibitors may be plagued by adverse effects as specific *jnk* mouse knockout models spontaneously develop intestinal tumours (Tong et al., 2007). Therefore, as long-term therapy is required to treat chronic inflammation, global inhibitors of JNK1 and p38 $\alpha$  by orally applied kinase inhibitors at this stage appear unlikely candidates (Gaestel et al., 2009).

Finally, some naturally occurring substances interact with the MAPK pathways and may be beneficial for endometriosis treatment alone, or in combination. Puerarin, a phytoestrogen, was shown to inhibit E2-BSA mediated proliferation, although not as strongly as the ERK inhibitor UO126 (Cheng et al., 2012). EGCG from green tea had a moderate effect on JNK phosphorylation with a concomitant effect on VEGF, which may mediate the angiogenic potential of endometriotic lesions (Xu et al., 2011). Artemisia leaves (APE) induced apoptosis of I2Z and I1Z endometriotic epithelial cells, which could be attenuated by the specific p38 inhibitor, SB203580 (Kim et al., 2013).

#### Summary

The MAPK pathways represent a series of pathways and interconnecting kinases that are influenced by the endometriotic microenvironment. The strongest evidence for constitutive activity in endometriotic tissue is available for ERK; however, this may simply be due to it being the most extensively studied. Importantly, all three pathways are influenced not only by inflammation, but also by oxidative stress and hormones. It is also possible that the MAPK pathways, and in particular JNK, have a significant role in the feedback mechanisms that limit the overexpression of other pathways activated in the endometriotic environment and thus combination targeting could be considered. Current strategies for targeting this pathway have focused on upstream

molecules, but appear associated with significant side effects that are not tolerable for endometriosis treatment. Downstream targeting of kinases that are dysregulated in endometriosis may reduce the adverse effects; however, for the p38 and JNK pathways liver toxicity and other side effects may represent a problem. Therefore in conclusion, while a dysregulation of this pathway in endometriotic microenvironment may occur, more specific targeting is required.

## The PI3K/AKT/mTOR pathway in endometriosis

The PI3K/Akt/mTOR pathway regulates cell growth, proliferation, differentiation and apoptosis in response to both intra- and extracellular signals including nutrients, energy and oxygen levels, inflammation and growth factors (Hennessy *et al.*, 2005). mTOR exists as either the mTOR complex 1 (mTORC1) or complex 2 (mTORC2). In mTORC1, the most extensively studied complex, mTOR is bound to four additional proteins; regulatory-associated protein of mTOR (raptor), mammalian lethal with Sec13 protein 8 (mLST8), proline rich AKT substrate (PRAS40) and DEP-domain-containing mTOR interacting protein (Deptor) and represents an important nodal point in this pathway. Upstream, the most common mediator of mTOR activity is the membrane-bound phosphoinositol 3 kinase (PI3K), a membrane-bound phospholipid that together with AKT, forms the core of the PI3K/AKT/mTOR pathway (Fig. 4).

Stimulation of the PI3K/AKT/mTOR pathway begins once PI3K is activated leading to the phosphorylation of phosphatidylinositol-4,5-biphosphate (PIP2) to phosphatidylinositol-3,4,5 triphosphate (PIP3). Proteins with a pleckstrin homology domain, such as phosphoinositide-dependent kinase 1 (PDK1) and AKT are co-recruited to PIP3 and their subsequent proximity results in AKT phosphorylation by PDK1 (Cantley, 2002). Phosphatase and tensin homolog deleted on chromosome ten (PTEN) functions as a negative regulator of this reaction by dephosphorylating PIP3, back to PIP2. Once phosphorylated, AKT subsequently regulates downstream activation of mTOR via an interaction with tuberin sclerosis complex (TSC)2 (Manning, 2004). TSC2 exists as a heterodimer with TSC1 and this complex is a negative regulator of mTOR activity through their interaction with GTPase Ras homology enriched in brain (Rheb) (Li *et al.*, 2004). Downstream targets for mTOR are predominantly proteins involved in the translational machinery and ribosomal recruitment to mRNA (Hay and Sonenberg, 2004; Fig. 4).

Crosstalk with other kinases is common in the PI3K/AKT/mTOR pathway. IKK $\beta$  interacts with TSC2 and influences mTOR mediated protein synthesis (Lee *et al.*, 2007; Fig. 4) and conversely AKT can influence both IKK $\beta$  and phosphorylate the p65 subunit of NF $\kappa$ B (Nidai Ozes *et al.*, 1999; Sizemore *et al.*, 1999). Interactions are also possible between the PI3K and MAPK pathways. The upstream mediator of the MAPK pathways, Ras-GTP, can bind and activate PI3K (Rodriguez-Viciana *et al.*, 1994) and an ERK mediated phosphorylation of TSC2 also occurs (Roux *et al.*, 2004). Importantly, however, these phosphorylation sites are different to that mediated by AKT phosphorylation. An interaction between p38 and mTOR has also been reported with the downstream target of p38 activation MK2, phosphorylating TSC2 at serine 1210 altering mTOR activity (Li *et al.*, 2003).

mTOR maintains cellular viability by striking a balance between the anabolic and catabolic processes, such as protein synthesis and autophagy. Protein synthesis is regulated through the activation of the mTOR substrates S6K and 4E-BP1, which translate a subset of messenger RNAs that promote cell growth and proliferation in a phospho-specific manner. When 4E-BP1 is dephosphorylated, it sequesters the eIF-4F cap-binding protein and inhibits its assembly into the eIF-4F cap-binding complex attenuating cap-dependent translation (Pause *et al.*, 1994). S6K is also able to mediate protein translation through multiple substrates, such as S6K1/aly/REF-like target (SKAR),

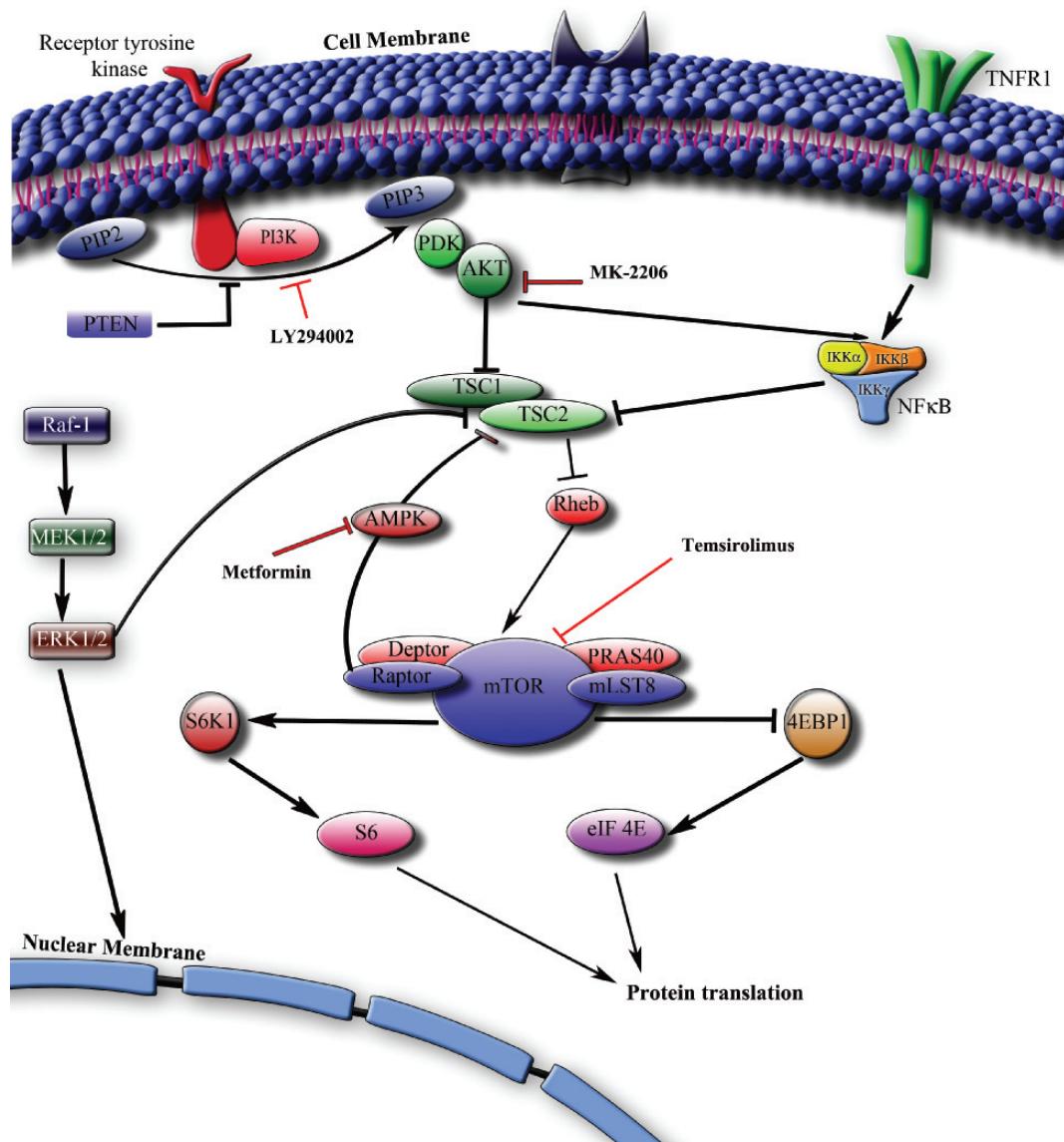
programmed cell death 4 (PCD4), eukaryotic initiation factor 4B (eIF4B) and ribosomal protein S6 (Ma and Blenis, 2009). Under growth promoting conditions, the S6 protein, a component of the 40S ribosomal unit, is primarily responsible for stimulating high rates of protein synthesis (Gressner and Wool, 1974).

Autophagy is a catabolic process whereby the cell liberates intracellular stores of nutrients by degrading cytoplasmic proteins in lysosomes. During periods where nutrition and growth factors are in abundance, mTOR inhibits autophagy. If nutrients and growth factors are withdrawn or oxidative stress occurs, inhibition of mTOR allows autophagic process to increase, resulting in the production of amino acids that function as a feedback loop to again activate mTOR and attenuate the autophagic response (Yu *et al.*, 2010). Given the presence of oxidative stress in the endometriotic microenvironment, the potential for activation of mTOR mediated autophagy should be an important consideration.

At present, little is known about the function of the PI3K/AKT/mTOR pathway in endometriosis, although there is some evidence of a dysregulation. Mutations in the PTEN gene have been identified in 21% of endometriomas (Sato *et al.*, 2000). Phosphorylated AKT has been observed in ovarian endometriosis of post-menopausal women (Yagyu *et al.*, 2006), and increased pAKT is present in eutopic and ectopic endometrial cells of women with endometriosis, compared with those from women without (Cinar *et al.*, 2009). An increased pAKT has also been observed in stromal cells from endometrioma compared with cells from the endometrium of women without endometriosis (Yin *et al.*, 2012). The over-activation of AKT may also lead to decreased PR expression in endometriosis (Eaton *et al.*, 2013). Phosphorylated mTOR is increased in ectopic lesions compared with the eutopic endometrium of women with endometriosis (Guo *et al.*, 2015) and increased mRNA expression of both AKT1 and 4EBP1 has also been observed in the eutopic endometrium of women with endometriosis compared with women without endometriosis (Laudanski *et al.*, 2009).

As a key regulator of the nutrient and growth factor levels, mTORC1 also contributes to glucose homeostasis, the regulation of iron-free radicals and oxidative stress. Although much of this work is still in its infancy, some relationships have been identified. Inhibition of PI3K/mTOR reduces the GLUT1 membrane localization in lung adenocarcinoma (Makinoshima *et al.*, 2015) and in cervical cancer the inhibition of AKT/mTOR significantly inhibits GLUT1 and GLUT4 membrane transport (Rashmi *et al.*, 2014). We have previously shown an altered regulation of GLUT1 and GLUT4 receptors in ectopic tissue (McKinnon *et al.*, 2014) and it is therefore possible that this may be mediated through a dysregulation in the mTOR mechanism, although it is yet to be investigated in endometriosis. mTOR has also recently been implicated in iron homeostasis (Bayeva *et al.*, 2012; Guan and Wang, 2014) and the modulation of iron uptake through regulation of the transferrin receptor (Galvez *et al.*, 2007). A dysregulation of the mTOR pathway in ectopic tissue could provide a means for iron overload within the endometriotic cells and a stimulation of oxidative stress.

Over-activation of the mTOR pathway may also be a function of the micro-environment. IL-8 increases AKT phosphorylation and the induction of the anti-apoptotic Bcl-2 and survivin proteins (Li *et al.*, 2012) in endometriotic stromal cells. In the immortalized epithelial 12Z cell line, TNF $\alpha$  stimulates AKT phosphorylation that is inhibited by wortmannin, a PI3K-specific inhibitor (Grund *et al.*, 2008) and 17 $\beta$ -E2 decreases PTEN expression in both normal and endometriotic cells (Zhang *et al.*, 2010a). In endometrial tissue from normal women, the menstrual cycle progression induces an autophagic response that does not occur in endometriotic women (Choi *et al.*, 2014) and markers of autophagy are increased in ovarian endometriomas, as is the oxidative marker heme oxygenase 1 (Allavena *et al.*, 2015). Platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and fibroblast growth factor 2 (FGF2) all stimulate a phosphorylation of AKT and cell migration in endometrial stromal cells (Gentilini *et al.*, 2007). Furthermore, the hyper-proliferative phenotype observed in DIE lesions is associated with



**Figure 4** The PI3K/AKT/mTOR signalling pathway and its inhibition in endometriosis. The mTOR pathway is activated by multiple extracellular stimuli through numerous cell membrane receptors, including receptor tyrosine kinases and cytokine receptors. Binding to these receptors stimulates PI3K to mediate the phosphorylation of PIP2 to PIP3, leading to an association between PDK and AKT. PTEN serves as an inhibitory protein in this reaction. The physical proximity between PDK and AKT leads to the phosphorylation of AKT and subsequent inhibition of TSC1. TSC1 exists as a heterodimer with TSC2 and through the Rheb GTPase has an inhibitory function against mTORC1, which exists in a complex with four additional proteins bound to mTOR, including Deptor, Raptor, PRAS40 and mLST8. Activation of mTORC1 leads to the activation of S6K1 and the downstream ribosomal S6 protein, as well as the inhibition of 4EBP1 that subsequently stimulates eIF4E and cap-dependent translation of mRNA and the translation of selected proteins. The mTOR pathway interacts with both the NF $\kappa$ B pathway and the ERK1/2 MAPK pathway through an interaction with TSC2. A negative feedback loop also via AMPK also connects mTOR with TSC2. Numerous pharmaceutical compounds modulate mTOR activity at different locations some of which have been trialled in endometriosis.

increased levels of endogenous oxidative stress and activation of the mTOR/AKT pathway (Leconte et al., 2011).

#### Targeting the PI3K/AKT/mTOR pathway in endometriosis

*In vitro* evidence indicates that disrupting the PI3K/mTOR pathway reduces the proliferation of endometriotic epithelial and stromal cells. Estrogen down-regulates nometastatic gene 23-H1 (NME1) expression, which mediates a subsequent elevation in expression of PCNA, survivin and integrin

(Li et al., 2013) as well as VEGF and IL-8 (Chang et al., 2013), all of which could be attenuated by LY294002, a specific PI3K inhibitor. Temsirolimus, a specific mTOR inhibitor, blocked proliferation of endometriotic cell proliferation *in vitro* and in a heterologous nude mouse model (Leconte et al., 2011). The inhibition of AKT phosphorylation by MK-2206 in stromal cells reduced the levels of a target protein p(S256)-forkhead box O1 and decreased the viability of cells from women both with and without endometriosis (Kim et al., 2014) (Fig. 4).

Some compounds already in use also exert off-target effects on mTOR pathway regulation. Metformin, an oral anti-diabetic drug (Stumvoll *et al.*, 1995) activates 5' adenosine monophosphate-activated protein kinase (AMPK), mediating the drug's effects in muscle, adipose, liver (Zhou *et al.*, 2001) and breast cancer cells (Zakikhani *et al.*, 2006). AMPK is a negative upstream regulator of TSC2, which exerts inhibitory effects on mTORC1 (Inoki *et al.*, 2003). A recent clinical study on metformin in endometriosis found a significant reduction in the symptomatic cases, increased chance of pregnancy, and a decrease in the levels of serum cytokines, suggesting an anti-endometriotic potential (Foda and Aal, 2012). Other studies had previously documented this treatment effect in rat models (Oner *et al.*, 2010; Yilmaz *et al.*, 2010).

A principle drawback of targeting the mTOR pathway is that the substantial crosstalk, as well as critical roles performed by this pathway increases the likelihood of unwanted side effects. The mTOR inhibitor temsirolimus, which has shown promise in reducing endometriotic lesions in *in vitro* and animal models (Leconte *et al.*, 2011), is currently approved for treatment of renal cell carcinoma and through this use, the class-specific toxicities of these drugs are emerging. Adverse effects commonly include an impact on the haematological, pulmonary and dermatological systems (Hutson *et al.*, 2008; Eisen *et al.*, 2012) and while these can be unpleasant they can be medically managed with close patient monitoring and early intervention with a return to normal after cessation of therapy (Bellmunt *et al.*, 2008). However, the immunosuppressive effects of temsirolimus have also been linked to an increase in infection of cancer patients (Kaymakcalan *et al.*, 2013) that one study linked to an increase in fatal adverse effects (Choueiri *et al.*, 2013). Similar to MAPK inhibitors, there is a suggestion that this class of drugs may be teratogenic, although limited evidence has been obtained due to ethical concerns. Whether these adverse effects and the need for their medical management have a sufficiently limited impact to warrant the use of temsirolimus in a non-life threatening condition, such as endometriosis, will need to be carefully considered against the symptomology of the patient, the technical difficulty of surgical removal of the endometriotic lesion and the patients' response to traditional therapies (Table I).

### Summary

The mTOR pathway plays a significant role in integrating signals from the extracellular environment into cell viability and proliferation and a number of kinases within this pathway may be over-active in endometriotic cells. This pathway therefore represents a potential treatment option for endometriosis. At present, however, even though there are numerous compounds that modulate this pathway, only a few of these have been trialled in endometriosis. While unwanted side effects still occur, the majority of these are non-life threatening, medically manageable and dissipate after cessation of treatment, particularly for temsirolimus. Therefore, although at present there are no clinical trials currently underway, they may have significant potential if their class-specific toxicities can be better delineated.

## Conclusion

Endometriosis treatment represents a complex clinical challenge and new therapies are needed. The peritoneal environment of endometriotic women is significantly altered which can lead to an over-activation of kinase signalling pathways in endometriotic tissue. In this manuscript, we reviewed three pathways: NF $\kappa$ B, MAPK and PI3K/AKT/mTOR in endometriotic cells. Increased activity of the NF $\kappa$ B pathway in endometriotic cells and *in vitro* and animal data supports its potential as a target. Less data were available on the MAPK pathway activation, although targeting ERK may have potential. Similarly, the PI3K/AKT/mTOR pathway also displays promising *in vitro* results in an endometriosis models. There is

therefore the potential for targeting these and perhaps other pathways in endometriosis if current limitations and challenges can be overcome.

### Limitations and challenges

Although an increase in the activity of many kinases in endometriotic cells has been identified, a specific kinase dependency for endometriotic lesions, through an activating genetic mutation is yet to be confirmed. It is possible, however, that a kinase dependency may stem from the extracellular environment. Kinase-dependent tumours without activating mutations, but with an overexpression of kinase ligands have previously been identified (Simon *et al.*, 1997; Shimizu *et al.*, 1999), as has the influence of the extracellular environment on the clinical efficacy of kinases targeting drugs (Jäne et al., 2009). Identifying the kinase dependency for endometriosis will be key to creating an effective kinase inhibiting therapeutic.

A lack of a specific, single kinase dependency may also present challenges in regards to acquired drug resistance. Tumour cells are adept at creating drug resistance by inducing mutations in other kinase signalling pathways when challenged (Zhang *et al.*, 2009). The ability of the extracellular environment to stimulate multiple signalling pathways could mean the extracellular environment has multiple possibilities to mediate tumour growth and that targeting a specific kinase will result in the over-activation of a compensatory pathway. Therefore, to successfully treat endometriosis through inhibition of these pathways, more information on kinase activation, the extracellular environment in endometriosis and the effects of interrupting this interaction is needed.

Management of the associated toxicity profiles is the most immediate challenge presented by the use of these drugs with both on-target and off-target effects responsible for their toxicity. Off-target effects are inherent to the high degree of conservation of the ATP biding sites across the human kinome, whereas the on-target effects are due to the central role these kinases play and are cell specific. The off-target effects may be addressed by drug design strategies and improved binding site specificities in next generation kinase inhibitors. Careful selection of dosage is also critical. The specificity of kinase inhibitors decreases as concentrations increase and there is little justification for concentrations above those required for maximal inhibition of the specific target, a consideration that should also be important during both *in vitro* and clinical studies. On-target effects present a more significant problem and will need to be assessed from a disease-specific point of view and thus more studies in endometriosis-specific models are needed.

### Future directions

While the adverse effects associated with these drugs limits their usefulness in endometriosis at present, well-designed clinical strategies could open the door to their clinical use in the future. As recently proposed by Santulli *et al.* (2015a, b) for MAPK inhibitors, the current generation of drugs could find a use in more severe cases of symptomatic DLE lesions (Santulli *et al.*, 2015b). These lesions have extracellular environments that predispose them to increase kinase activation are more likely resistant to hormonal modulation and represent complicated surgical procedures. If proven to be cytoreductive, these drugs could be used for short-term treatment prior to surgery to reduce the size and depth of a lesion. Furthermore, women with strong symptoms may also be more willing to tolerate the adverse effects short-term. An important consideration, however, is the potential embryotoxic and

**Table I** Pharmaceutical compounds that interact with kinase signalling pathways and trialled in endometriosis treatment.

Signalling pathway	Compound	Iupac name	Target	Function	Reference	Functional target validation
NF $\kappa$ B	BAY 11-7085	(E)-3-(4-tert-butylphenyl)sulfonylprop-2-enenitrile <sup>b</sup>	I $\kappa$ B	Inhibits I $\kappa$ B phosphorylation Decreases cell proliferation and DNA synthesis. Induces apoptosis	Pierce <i>et al.</i> (1997) Nasu <i>et al.</i> (2007)	Endometriotic stromal cells
				Decreases lesion size Increases apoptotic markers.	González-Ramos <i>et al.</i> (2008)	Heterologous nude mouse model
BORTEZOMIB		Mannitol boronic ester, [(IR)-3-methyl-1-[[[(2S)-3-phenyl-2-(pyrazine-2-carbonylamino)propanoyl]amino]butyl]boronic acid <sup>b</sup>	Proteasome	Reduces endometriotic lesion size Decreases PCNA and Ki67 expression	Celik <i>et al.</i> (2008)	Transplanted endometriosis in Wistar Rat
TPCK		N-Tosyl-L-Phenylalanine Chloromethyl ketone, N-[(2S)-4-chloro-3-oxo-1-phenylbutan-2-yl]-4-methylbenzenesulfonamide <sup>b</sup>	NF $\kappa$ B	Anti-NF $\kappa$ B activity	Yamauchi <i>et al.</i> (2004)	Endometrioma stromal cells
PDTc		Pyrrolidine dithiocarbamate, 2-acetamido-3-sulfanylpropanoic acid;pyrrolidine-1-carbodithioic acid <sup>b</sup>	I $\kappa$ B	I $\kappa$ B-ubiquitin ligase	Hayakawa <i>et al.</i> (2003)	Jurkat T-cells
			NA	Decreases inflammation, angiogenic factors and MMPs	Zhang <i>et al.</i> (2010a, b)	Endometriotic stromal cells
			NA	Reduces in lesion size	Zhang <i>et al.</i> (2011)	Endometriotic epithelial cells
THALIDOMIDE		$\alpha$ -Phthalimido glutarimide, 2-(2,6-dioxopiperidin-3-yl)isoindole-1,3-dione <sup>b</sup>	I $\kappa$ B	Suppression of I $\kappa$ B degradation	Majumdar <i>et al.</i> (2002)	Transplanted endometriosis in Wistar Rats
			NF $\kappa$ B	Inhibits TNF $\alpha$ -stimulated IL-8	Yagyu <i>et al.</i> (2005)	NA
			NA	Reduces endometrial implants	Azimirad <i>et al.</i> (2014)	Autologous endometrial implant in Sprague-Dawley rat
THIAZOLIDINEDIONES		1,3-Thiazolidine-2,4-dione <sup>b</sup>	PPAR $\gamma$	Reduces endometriotic lesion size	Lebovic <i>et al.</i> (2004)	Autologous endometrial implant in Sprague-Dawley rat
					Lebovic <i>et al.</i> (2007)	Primates
CELECOXIB (NSAID)		4-[5-(4-Methylphenyl)-3-(trifluoromethyl)pyrazol-1-yl]benzenesulfonamide <sup>b</sup>	COX-2	Interacts with NF $\kappa$ B Decreases cellular proliferation	Park <i>et al.</i> (2014)	Leiomyoma cells
SULINDAC (NSAID)		2-[(3Z)-6-fluoro-2-methyl-3-[(4-methylsulfinylphenyl)methylidene]inden-1-yl]acetic acid <sup>b</sup>	NA	Decreases RANTES through NF $\kappa$ B mechanism	Olivares <i>et al.</i> (2008) Wieser <i>et al.</i> (2005), Efstatouli <i>et al.</i> (2005)	Endometrial epithelial cells
RESVERATROL <sup>a</sup>		3,5,4'-Trihydroxy-trans-stilbene, 5-[(E)-2-(4-hydroxyphenyl)ethenyl]benzene-1,3-diol <sup>b</sup>	NA	Decreases RANTES through NF $\kappa$ B mechanism	Wieser <i>et al.</i> (2005), Efstatouli <i>et al.</i> (2005)	Normal and endometriotic stromal cells
			NA	Reduces surgically induced endometriotic lesions	Bruner-Tran <i>et al.</i> (2011)	C57BL/6j mice
						Nude (NCr) mice

Continued

**Table I** Continued

Signalling pathway	Compound	Iupac name	Target	Function	Reference	Functional target validation
	EGCG <sup>a</sup>	Epigallocatechin-3-gallate, [(2R,3R)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-chromen-3-yl] 3,4,5-trihydroxybenzoate <sup>b</sup>	NA	Reduces cell proliferation Increases apoptosis	Ricci <i>et al.</i> (2013), Rudzitis-Auth <i>et al.</i> (2013)	Endometrial epithelial cells
	PARTHENOLIDE <sup>a</sup>	(1aR,7aS,10aS,10bS)-1a,5-dimethyl-8-methylene-2,3,6,7,7a,8,10a,10b-octahydrooxireno[9,10]cyclodeca[1,2-b]furan-9(1aH)-one	NA	Reduces cytokine concentrations	Ergenoglu <i>et al.</i> (2013), Ozcan Cenksoy <i>et al.</i> (2015), Bayoglu Tekin <i>et al.</i> (2015)	Peritoneal from Rat models
	CURCUMIN <sup>a</sup>	(1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione <sup>b</sup>	NA	Interacts with NF $\kappa$ B Reduces surgically induced endometriotic lesions	Khan <i>et al.</i> (2006) Ricci <i>et al.</i> (2013)	NA mice
	IL-10	Interleukin 10	NA	Inhibits NF $\kappa$ B activity Reduces the inflammatory response	Kwok <i>et al.</i> (2001) Takai <i>et al.</i> (2013)	NA
	Decoy Nucleotides	Nucleotide sequences Forward; 5'-CCTTGAAGGGATTTC CCTCC-3' Reverse; 3'-GGAACCTTCCCTAAAGGGAGG-5'	NA	Attenuates cytokine secretion and inflammation	Veillat <i>et al.</i> (2009), Kim <i>et al.</i> (2012), Jana <i>et al.</i> (2012)	Endometriotic stromal cells BALB/c mice
MAPK	VEMURAFENIB	N-[3-[5-(4-chlorophenyl)-1H-pyrrolo[2,3-b]pyridine-3-carbonyl]-2,4-difluorophenyl]propane-1-sulfonamide <sup>b</sup>	B-raf	NA	Su <i>et al.</i> (2012)	Approved for use in melanoma
	DABRAFENIB	N-[3-[5-(2-aminopyrimidin-4-yl)-2-tert-butyl-1,3-thiazol-4-yl]-2-fluorophenyl]-2,6-difluorobenzenesulfonamide <sup>b</sup>	NA			
	TRAMETINIB	N-(3-[3-Cyclopropyl-5-[(2-fluoro-4-iodophenyl)amino]-6,8-dimethyl-2,4,7-trioxa-3,4,6,7-tetrahydropyrido[4,3-d]pyrimidin-1(2H)-yl]phenyl)acetamide <sup>b</sup>	MEK	NA	Menzies <i>et al.</i> (2015)	NA
	Sorafenib	4-[4-[[4-Chloro-3-(trifluoromethyl)phenyl]carbamoylamino]phenoxy]-N-methylpyridine-2-carboxamide	Raf-1, B-Raf, VEGFR 1, 2, 3, PDGFR- $\beta$ , c-KIT	Inhibits cellular proliferation. Decreases lesion size	Leconte <i>et al.</i> (2015)	Endometrial stromal cells Nude mouse model
	GW5074	(3Z)-3-[(3,5-dibromo-4-hydroxyphenyl)methylidene]-5-iodo-1H-indol-2-one <sup>b</sup>	Raf-1	Inhibits cell proliferation and invasion	De La Garza <i>et al.</i> (2012)	Epithelial EM42 cells and primary stromal cells
	A771726	(Z)-2-cyano-3-hydroxy-N-[4-(trifluoromethyl)phenyl]but-2-enamide <sup>b</sup>	ERK	Decrease cell proliferation	Leconte <i>et al.</i> (2011)	Epithelial and stromal cells from eutopic and ectopic lesions
	UO126	(2Z,3Z)-2,3-bis[amino-(2-aminophenyl)sulfonylmethylidene]butanedinitrile <sup>b</sup>	NA		Matsuzaki and Darcha (2015)	
	PD98059	2-(2-Amino-3-methoxyphenyl)-4H-chromen-4-one <sup>b</sup>	NA		Ngó <i>et al.</i> (2010)	
	FRI180204	5-(2-Phenylpyrazolo[1,5-a]pyridin-3-yl)-2H-pyrazolo[3,4-c]pyridazin-3-amine <sup>b</sup>	NA	Alleviate clinical arthritis	Ohori <i>et al.</i> (2007)	DBA/1 mouse model

SCH772984	(3R)-1-[2-oxo-2-[4-(4-pyrimidin-2-ylphenyl)piperazin-1-yl]ethyl]-N-(3-pyridin-4-yl-1H-indazol-5-yl)pyrrolidine-3-carboxamide <sup>b</sup>		NA		Morris <i>et al.</i> (2013)	Preclinical testing in BRAF and MEK inhibitor-resistant cell lines
FR167653	I-[7-(4-Fluorophenyl)-1,2,3,4-tetrahydro-8-(4-pyridyl)pyrazolo[5,1-c][1,2,4]triazin-2-yl]-2-phenylethanedione sulphate monohydrate	p38	Reduces endometriotic lesion size. Reduces peritoneal fluid IL-6 and MCP-1		Yoshino <i>et al.</i> (2006)	BALB/c mice
SB203580	4-[4-(4-Fluorophenyl)-2-(4-methylsulfinylphenyl)-1H-imidazol-5-yl]pyridine <sup>b</sup>		Reduces IL-1 $\beta$ secretion and endometriotic lesion size	Huang <i>et al.</i> (2013)	Endometriotic stromal cells	
SB202190	4-[4-(4-Fluorophenyl)-5-pyridin-4-yl-1,3-dihydroimidazol-2-ylidene]cyclohexa-2,5-dien-1-one <sup>b</sup>		Reducing TNF $\alpha$ , IL-1 $\beta$ , MMP3 and MMP9 mRNA and protein concentrations	Zhou <i>et al.</i> (2010)	BALB/c mice; cells isolated from peritoneal cavity	
VX-745	5-(2,6-Dichlorophenyl)-2-(2,4-difluorophenyl)sulfanylpyrimido[1,6-b]pyridazin-6-one <sup>b</sup>		Attenuates cell proliferation	OuYang <i>et al.</i> (2008)	Endometriotic stromal cells	
BIRB 796	Doramapimod, 1-[5-tert-butyl-2-(4-methylphenyl)pyrazol-3-yl]-3-[4-(2-morpholin-4-ylethoxy)naphthalen-1-yl]urea <sup>b</sup>		NA	Dambach (2005)	Failed phase II clinical trials due to high liver toxicity	
SP600125	1,9-Pyrazoloanthrone, Dibenzo[cd,g]indazol-6(2H)-one	JNK	Reducing the inflammatory response	Han <i>et al.</i> (2001)	Mouse models and human synoviocytes as a model of rheumatoid arthritis	
PGL5001	Doramapimod				Yoshino <i>et al.</i> (2004)	Endometriotic stromal cells
Puerarin <sup>a</sup>	7-Hydroxy-3-(4-hydroxyphenyl)-8-[(2S,3R,4R,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]chromen-4-one <sup>b</sup>	NA	Inhibits E2-BSA mediated proliferation	Cheng <i>et al.</i> (2012)	Phase IIa clinical trial in the treatment of endometriosis	
ECGC <sup>a</sup>	Epigallocatechin-3-gallate, [(2R,3R)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-chromen-3-yl] 3,4,5-trihydroxybenzoate <sup>b</sup>	JNK	Moderate effect on JNK phosphorylation and VEGFC	Xu <i>et al.</i> (2011)	Endometriotic stromal cells	
Artemisia leaves <sup>a</sup>	NA	NA	Induces apoptosis	Kim <i>et al.</i> (2013)	Heterologous mouse models	
KT/ LY294002	2-Morpholin-4-yl-8-phenylchromen-4-one <sup>b</sup>	PI3K	Induces apoptosis	Li <i>et al.</i> (2013), Chang <i>et al.</i> (2013)	Endometrial stromal cells	
TEMSIROLIMUS	(1R,2R,4S)-4-[(2R)-2-[(3S,6R,7E,9R,10R,12R,14S,15E,17E,19E,21S,23S,26R,27R,34aS)-9,27-dihydroxy-10,21-dimethoxy-6,8,12,14,20,26-hexamethyl-1,5,11,28,29-pentaoxo-1,4,5,6,9,10,11,12,13,14,21,22,23,24,25,26,27,28,29,31,32,33,34,34a-tetracosahydro-3H-23,27-epoxypyrido[2,1-c][1,4]oxazacycloheptatriacontin-3-yl]propyl]-2-methoxycyclohexyl 3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate <sup>b</sup>	mTOR	Decreases PCNA, surviving, integrin, VEGF and IL-8 expression	Leconte <i>et al.</i> (2011)	Heterologous nude mouse model	
			Inhibits proliferation		Behbakht <i>et al.</i> (2011)	Endometriotic cells <i>in vitro</i>
						Phase II trial in ovarian cancer patients

*Continued*

Signalling pathway	Compound	Iupac name	Target	Function	Reference	Functional target validation
MK-2206	8-[4-(1-Aminocyclobutyl)phenyl]-9-phenyl-2H-[1,2,4]triazolo[1,6]naphthyridin-3-one <sup>b</sup>	AKT	Reduces FOXO1 protein. Decreases cell viability	Kim <i>et al.</i> (2014)		Endometriosis and endometrial stromal cells
METFORMIN	3-(Diaminomethylidene)-1,1-dimethylguanidine <sup>b</sup>	NA	Activates AMPK kinase. Increased chances of pregnancy. Decreases serum cytokines	Zhou <i>et al.</i> (2001) Zakikhani <i>et al.</i> (2006) Foda and Aal (2012)	Onur <i>et al.</i> (2010), Yilmaz <i>et al.</i> (2010)	Muscle, adipose, liver Breast cancer cells Endometriosis patients Wistar rat model

<sup>a</sup>Natural compounds.  
<sup>b</sup>IUPAC name.

teratogenic effects of these compounds and thus treatments should be performed in combination with contraception allowing for at least 6 months post therapy wash-out. It should also be noted that due to the influence on CYP3A4-mediated metabolism, the plasma concentrations of hormonal contraceptives could vary and caution on their effectiveness during this period should be considered.

Future treatment strategies for kinase inhibitors could also incorporate the heterogeneity of endometriosis and target-specific kinases based on individual patient profiles. Through the use of robust and reproducible genome wide association studies, the genetic basis of endometriosis is increasingly being elucidated (Rahmioglu *et al.*, 2014) as are the peripheral changes and the extracellular environment that influence the disease progression and symptomology (Morotti *et al.*, 2014; McKinnon *et al.*, 2015). A better understanding of their biochemical basis and inflammatory profiles of endometriotic subtypes and the contribution of specific kinase pathways to individual endometriotic lesions may soon provide more information on the kinase dependency of specific lesions and the opportunity for personalized treatment.

Endometriosis research is gradually advancing the understanding of the disease pathogenesis; the task now is to translate these discoveries into novel therapeutics. An over-activation of kinases in endometriotic tissue has been observed and thus the targeting of kinase signalling pathways represents a valid treatment option. In the near future these drugs may find applications for short-term use in more severe cases, but at present more information is needed on the dysregulation of these pathways in endometriotic tissue. Looking further ahead the outlook is promising, early studies suggest these drugs can be cytoreductive and the development of new kinase inhibitors is increasing and thus so is the likelihood of improvements in their specificity and side effects profiles. A reduction in the adverse effects, combined with more knowledge on which patients to match to particular drugs through an understanding of endometriosis heterogeneity and kinase dependency could make them tolerable and efficacious for endometriosis patients.

## Authors' roles

B.D.M. conceived, designed and prepared the manuscript and figures. V.K. contributed to section about biological bases of signalling pathways. K.N. contributed to clinical and treatment sections. N.A.B. contributed to section on the endometriotic microenvironment. M.D.M contributed to the concept, intellectual content and revision the manuscript.

## Funding

This review was supported by the Swiss National Science Foundation (320030\_140774).

## Conflict of interest

All authors declare there are no conflicts of interest.

## References

Abrão MS, Petraglia F, Falcone T, Keckstein J, Osuga Y, Chapron C. Deep endometriosis infiltrating the recto-sigmoid: critical factors to consider before management. *Hum Reprod Update* 2015;21:329–339.

Adler V, Franklin CC, Kraft AS. Phorbol esters stimulate the phosphorylation of c-Jun but not v-Jun: regulation by the N-terminal delta domain. *Proc Natl Acad Sci USA* 1992;89:5341–5345.

Allavena G, Carrarelli P, Del Bello B, Luisi S, Petraglia F, Maellaro E. Autophagy is upregulated in ovarian endometriosis: a possible interplay with p53 and heme oxygenase-1. *Fertil Steril* 2015;103:1244–1251.e1.

Alvarado-Díaz CP, Núñez MT, Devoto L, González-Ramos R. Iron overload-modulated nuclear factor kappa-B activation in human endometrial stromal cells as a mechanism postulated in endometriosis pathogenesis. *Fertil Steril* 2015;103:439–447.

Amorim MHR, Gil da Costa RM, Lopes C, Bastos MMSM. Sesquiterpene lactones: adverse health effects and toxicity mechanisms. *Crit Rev Toxicol* 2013;43:559–579.

Andrade SS, de Azevedo AC, Monasterio ICG, Paredes-Gamero EJ, Gonçalves GA, Bonetti TC, Albertoni G, Schor E, Barreto JA, Luiza Oliva M et al. 17 $\beta$ -Estradiol and steady-state concentrations of H<sub>2</sub>O<sub>2</sub>: antiapoptotic effect in endometrial cells from patients with endometriosis. *Free Radic Biol Med* 2013;60:63–72.

Arumugam K, Yip YC. De novo formation of adhesions in endometriosis: the role of iron and free radical reactions. *Fertil Steril* 1995;64:62–64.

Asante A, Taylor RN. Endometriosis: the role of neuroangiogenesis. *Annu Rev Physiol* 2011;73:163–182.

Azimirad A, Alborzi S, Kumar PV, Zolghadri J, Zarei A, Tavana Z, Azimirad M. Thalidomide affects experimental endometriosis: a randomized controlled study in the rat. *J Obstet Gynaecol Res* 2014;40:1989–1997.

Azzolina A, Bongiovanni A, Lampiasi N. Substance P induces TNF-alpha and IL-6 production through NF kappa B in peritoneal mast cells. *Biochim Biophys Acta* 2003;1643:75–83.

Badawy SZ, Marshall L, Cuenca V. Peritoneal fluid prostaglandins in various stages of the menstrual cycle: role in infertile patients with endometriosis. *Int J Fertil* 1985;30:48–52.

Bayeva M, Khechaduri A, Puig S, Chang H-C, Patial S, Blackshear PJ, Ardehali H. mTOR regulates cellular iron homeostasis through tristetraprolin. *Cell Metab* 2012;16:645–657.

Bayoglu Tekin Y, Guven S, Kirbas A, Kalkan Y, Tumkaya L, Guvendag Guven ES. Is resveratrol a potential substitute for leuproide acetate in experimental endometriosis? *Eur J Obstet Gynecol Reprod Biol* 2015;184:1–6.

Bebakht K, Sill MW, Darcy KM, Rubin SC, Mannel RS, Waggoner S, Schilder RJ, Cai KQ, Godwin AK, Alpaugh RK. Phase II trial of the mTOR inhibitor, temsirolimus and evaluation of circulating tumor cells and tumor biomarkers in persistent and recurrent epithelial ovarian and primary peritoneal malignancies: a Gynecologic Oncology Group study. *Gynecol Oncol* 2011;123:19–26.

Bellmunt J, Szczylik C, Feingold J, Strahs A, Berkenblit A. Temsirolimus safety profile and management of toxic effects in patients with advanced renal cell carcinoma and poor prognostic features. *Ann Oncol* 2008;19:1387–1392.

Bennett BL, Sasaki DT, Murray BW, O'Leary EC, Sakata ST, Xu W, Leisten JC, Motiwala A, Pierce S, Satoh Y et al. SP600125, an anthrapyrazolone inhibitor of Jun N-terminal kinase. *Proc Natl Acad Sci USA* 2001;98:13681–13686.

Bersinger NA, Frischknecht F, Taylor RN, Mueller MD. Basal and cytokine-stimulated production of epithelial neutrophil activating peptide-78 (ENA-78) and interleukin-8 (IL-8) by cultured human endometrial epithelial and stromal cells. *Fertil Steril* 2008;89:1530–1536.

Bersinger NA, Gunthert AR, McKinnon B, Johann S, Mueller MD. Dose-response effect of interleukin (IL)-1 $\beta$ , tumour necrosis factor (TNF)-alpha, and interferon-gamma on the in vitro production of epithelial neutrophil activating peptide-78 (ENA-78), IL-8, and IL-6 by human endometrial stromal cells. *Arch Gynecol Obstet* 2011;283:1291–1296.

Bertschi D, McKinnon BD, Evers J, Bersinger NA, Mueller MD. Enhanced inflammatory activity of endometriotic lesions from the rectovaginal septum. *Mediat Inflamm* 2013;2013:450950.

Bodmer M, Meier C, Kraenzlin ME, Meier CR. Risk of fractures with glitazones: a critical review of the evidence to date. *Drug Saf* 2009;32:539–547.

Bonizzi G, Karin M. The two NF-kappaB activation pathways and their role in innate and adaptive immunity. *Trends Immunol* 2004;25:280–288.

Brose MS, Nutting CM, Jarzab B, Elisei R, Siena S, Bastholt L, de la Fouchardiere C, Pacini F, Paschke R, Shong YK et al. Soraferib in radioactive iodine-refractory, locally advanced or metastatic differentiated thyroid cancer: a randomised, double-blind, phase 3 trial. *The Lancet* 2014;384:319–328.

Brown J, Farquhar C. Endometriosis: an overview of Cochrane Reviews. *Cochrane Database Syst Rev* 2014;3:CD009590.

Brunner-Tran KL, Osteen KG, Taylor HS, Sokalska A, Haines K, Duleba AJ. Resveratrol inhibits development of experimental endometriosis in vivo and reduces endometrial stromal cell invasiveness in vitro. *Biol Reprod* 2011;84:106–112.

Bulun SE, Cheng Y-H, Pavone ME, Xue Q, Attar E, Trukhacheva E, Tokunaga H, Utsunomiya H, Yin P, Luo X et al. Estrogen receptor-beta, estrogen receptor-alpha, and progesterone resistance in endometriosis. *Semin Reprod Med* 2010;28:36–43.

Burney RO, Giudice LC. Pathogenesis and pathophysiology of endometriosis. *Fertil Steril* 2012;98:511–519.

Cantley LC. The phosphoinositide 3-kinase pathway. *Science* 2002;296:1655–1657.

Cao W-G, Morin M, Metz C, Maheux R, Akoum A. Stimulation of macrophage migration inhibitory factor expression in endometrial stromal cells by interleukin 1, beta involving the nuclear transcription factor NFkappaB. *Biol Reprod* 2005;73:565–570.

Cao W-G, Morin M, Sengers V, Metz C, Roger T, Maheux R, Akoum A. Tumour necrosis factor-alpha up-regulates macrophage migration inhibitory factor expression in endometrial stromal cells via the nuclear transcription factor NF-kappaB. *Hum Reprod* 2006;21:421–428.

Carli C, Metz CN, Al-Abed Y, Naccache PH, Akoum A. Up-regulation of cyclooxygenase-2 expression and prostaglandin E2 production in human endometriotic cells by macrophage migration inhibitory factor: involvement of novel kinase signalling pathways. *Endocrinology* 2009;150:3128–3137.

Carvalho LFP, Samadder AN, Agarwal A, Fernandes LFC, Abrão MS. Oxidative stress biomarkers in patients with endometriosis: systematic review. *Arch Gynecol Obstet* 2012;286:1033–1040.

Castrillo A, Diaz-Guerra MJ, Hortelano S, Martin-Sanz P, Bosca L. Inhibition of IkappaB kinase and IkappaB phosphorylation by 15-deoxy-Delta(12,14)-prostaglandin J(2) in activated murine macrophages. *Mol Cell Biol* 2000;20:1692–1698.

Celik O, Hascalik S, Elter K, Tagluk ME, Gurates B, Aydin NE. Combating endometriosis by blocking proteasome and nuclear factor-kappaB pathways. *Hum Reprod* 2008;23:2458–2465.

Chang K-K, Liu L-B, Jin L-P, Meng Y-H, Shao J, Wang Y, Mei J, Li M-Q, Li D-J. NME1 suppression of endometrial stromal cells promotes angiogenesis in the endometriotic milieu via stimulating the secretion of IL-8 and VEGF. *Int J Clin Exp Pathol* 2013;6:2030–2038.

Chang K-K, Liu L-B, Li H, Mei J, Shao J, Xie F, Li M-Q, Li D-J. TSLP induced by estrogen stimulates secretion of MCP-1 and IL-8 and growth of human endometrial stromal cells through JNK and NF- $\kappa$ B signal pathways. *Int J Clin Exp Pathol* 2014;7:1889–1899.

Chapron C, Fauconnier A, Dubuisson JB, Barakat H, Vieira M, Bréart G. Deep infiltrating endometriosis: relation between severity of dysmenorrhoea and extent of disease. *Hum Reprod* 2003a;18:760–766.

Chapron C, Fauconnier A, Vieira M, Barakat H, Dousset B, Pansini V, Vacher-Lavenu MC, Dubuisson JB. Anatomical distribution of deeply infiltrating endometriosis: surgical implications and proposition for a classification. *Hum Reprod* 2003b;18:157–161.

Chapron C, Pietrin-Vialle C, Borghese B, Davy C, Foulot H, Chopin N. Associated ovarian endometrioma is a marker for greater severity of deeply infiltrating endometriosis. *Fertil Steril* 2009;92:453–457.

Chapron C, Bourret A, Chopin N, Dousset B, Leconte M, Amselle-Ouazana D, de Ziegler D, Borghese B. Surgery for bladder endometriosis: long-term results and concomitant management of associated posterior deep lesions. *Hum Reprod* 2010;25:884–889.

Chen S, Wu R-F, Su L, Zhou W-D, Zhu M-B, Chen Q-H. Lipoxin A4 regulates expression of the estrogen receptor and inhibits 17 $\beta$ -estradiol induced p38 mitogen-activated protein kinase phosphorylation in human endometriotic stromal cells. *Fertil Steril* 2014;102:264–271.

Chen Z, Xie F, Bao M, Li X, Chao Y, Lin C, Guo R, Zhang C, Wu A, Yue Y et al. Activation of p38 MAPK in the rostral ventromedial medulla by visceral noxious inputs transmitted via the dorsal columns may contribute to pelvic organ cross-sensitization in rats with endometriosis. *Neuroscience* 2015;291:272–278.

Cheng W, Chen L, Yang S, Han J, Zhai D, Ni J, Yu C, Cai Z. Puerarin suppresses proliferation of endometriotic stromal cells partly via the MAPK signaling pathway induced by 17 $\beta$ -estradiol-BSA. *PLoS One* 2012;7:e45529.

Cho YJ, Park SB, Han M. Di-(2-ethylhexyl)-phthalate induces oxidative stress in human endometrial stromal cells in vitro. *Mol Cell Endocrinol* 2015;407:9–17.

Choi J, Jo M, Lee E, Kim HJ, Choi D. Differential induction of autophagy by mTOR is associated with abnormal apoptosis in ovarian endometriotic cysts. *Mol Hum Reprod* 2014;20:309–317.

Choueiri TK, Je Y, Sonpavde G, Richards CJ, Galsky MD, Nguyen PL, Schutz F, Heng DY, Kaymakcalan MD. Incidence and risk of treatment-related mortality in cancer patients treated with the mammalian target of rapamycin inhibitors. *Ann Oncol* 2013; **24**:2092–2097.

Cinar O, Seval Y, Uz YH, Cakmak H, Ulukus M, Kayisli UA, Arici A. Differential regulation of Akt phosphorylation in endometriosis. *Reprod Biomed Online* 2009; **19**:864–871.

Dambach DM. Potential adverse effects associated with inhibition of p38alpha/beta MAP kinases. *Curr Top Med Chem* 2005; **5**:929–939.

Daynes RA, Jones DC. Emerging roles of PPARs in inflammation and immunity. *Nat Rev Immunol* 2002; **2**:748–759.

De La Garza EM, Binkley PA, Ganapathy M, Krishnegowda NK, Tekmal RR, Schenken RS, Kirma NB. Raf-I, a potential therapeutic target, mediates early steps in endometriosis lesion development by endometrial epithelial and stromal cells. *Endocrinology* 2012; **153**:3911–3921.

Defrère S, Lousse JC, González-Ramos R, Colette S, Donnez J, Van Langendonck A. Potential involvement of iron in the pathogenesis of peritoneal endometriosis. *Mol Hum Reprod* 2008; **14**:377–385.

Dhillon AS, Hagan S, Rath O, Kolch W. MAP kinase signalling pathways in cancer. *Oncogene* 2007; **26**:3279–3290.

Duffy JMN, Arambage K, Correa FJS, Olive D, Farquhar C, Garry R, Barlow DH, Jacobson TZ. Laparoscopic surgery for endometriosis. *Cochrane Database Syst Rev* 2014; **4**:CD011031.

Dunselman GAJ, Vermeulen N, Becker C, Calhaz-Jorge C, D'Hooghe T, De Bie B, Heikinheimo O, Horne AW, Kiesel L, Nap A et al. ESHRE guideline: management of women with endometriosis. *Hum Reprod* 2014; **29**:400–412.

Eaton JL, Unno K, Caraveo M, Lu Z, Kim JJ. Increased AKT or MEK1/2 activity influences progesterone receptor levels and localization in endometriosis. *J Clin Endocrinol Metab* 2013; **98**:E1871–E1879.

Efstathiou JA, Sampson DA, Levine Z, Rohan RM, Zurakowski D, Folkman J, D'Amato RJ, Rupnick MA. Nonsteroidal antiinflammatory drugs differentially suppress endometriosis in a murine model. *Fertil Steril* 2005; **83**:171–181.

Eisen T, Sternberg CN, Robert C, Mulders P, Pyle L, Zbinden S, Izzidine H, Escudier B. Targeted therapies for renal cell carcinoma: review of adverse event management strategies. *J Natl Cancer Inst* 2012; **104**:93–113.

Ergenoglu AM, Yeniel AO, Erbaş O, Aktug H, Yildirim N, Ulukus M, Taskiran D. Regression of endometrial implants by resveratrol in an experimentally induced endometriosis model in rats. *Reprod Sci* 2013; **20**:1230–1236.

Eskenazi B, Warner ML. Epidemiology of endometriosis. *Obstet Gynecol Clin* 1997; **24**:235–258.

Ferrero S, Abbamonte LH, Parisi M, Ragni N, Remorgida V. Dyspareunia and quality of sex life after laparoscopic excision of endometriosis and postoperative administration of triptorelin. *Fertil Steril* 2007; **87**:227–229.

Foda AA, Aal IAA. Metformin as a new therapy for endometriosis, its effects on both clinical picture and cytokines profile. *Middle East Fertil Soc J* 2012; **17**:262–267.

Gaestel M, Kotlyarov A, Kracht M. Targeting innate immunity protein kinase signalling in inflammation. *Nat Rev Drug Discov* 2009; **8**:480–499.

Galvez T, Teruel MN, Heo WD, Jones JT, Kim ML, Liou J, Myers JW, Meyer T. siRNA screen of the human signaling proteome identifies the PtdIns(3,4,5)P3-mTOR signaling pathway as a primary regulator of transferrin uptake. *Genome Biol* 2007; **8**:R142.

Gentilini D, Busacca M, Di Francesco S, Vignali M, Viganò P, Di Blasio AM. PI3K/Akt and ERK1/2 signalling pathways are involved in endometrial cell migration induced by 17beta-estradiol and growth factors. *Mol Hum Reprod* 2007; **13**:317–322.

Ghosh S, Karin M. Missing pieces in the NF-kappaB puzzle. *Cell* 2002; **109** (Suppl): S81–S96.

Ghosh S, May MJ, Kopp EB. NF-kappa B and Rel proteins: evolutionarily conserved mediators of immune responses. *Annu Rev Immunol* 1998; **16**:225–260.

Gilmore TD, Herscovitch M. Inhibitors of NF-kappaB signaling: 785 and counting. *Oncogene* 2006; **25**:6887–6899.

Gonzalez-Ramos R, Donnez J, Defrère S, Leclercq I, Squifflet J, Lousse JC, Van Langendonck A. Nuclear factor-kappa B is constitutively activated in peritoneal endometriosis. *Mol Hum Reprod* 2007; **13**:503–509.

González-Ramos R, Van Langendonck A, Defrère S, Lousse J-C, Mettlen M, Guillet A, Donnez J. Agents blocking the nuclear factor-kappaB pathway are effective inhibitors of endometriosis in an in vivo experimental model. *Gynecol Obstet Invest* 2008; **65**:174–186.

González-Ramos R, Rocco J, Rojas C, Sovino H, Poch A, Kohen P, Alvarado-Díaz C, Devoto L. Physiologic activation of nuclear factor kappa-B in the endometrium during the menstrual cycle is altered in endometriosis patients. *Fertil Steril* 2012; **97**:645–651.

Gori I, Pellegrini C, Staedler D, Russell R, Jan C, Canni GO. Tumor necrosis factor- $\alpha$  activates estrogen signaling pathways in endometrial epithelial cells via estrogen receptor  $\alpha$ . *Mol Cell Endocrinol* 2011; **345**:27–37.

Gressner AM, Wool IG. The phosphorylation of liver ribosomal proteins in vivo. Evidence that only a single small subunit protein (S6) is phosphorylated. *J Biol Chem* 1974; **249**:6917–6925.

Grund EM, Kagan D, Tran CA, Zeitvogel A, Starzinski-Powitz A, Nataraja S, Palmer SS. Tumor necrosis factor-alpha regulates inflammatory and mesenchymal responses via mitogen-activated protein kinase kinase, p38, and nuclear factor kappaB in human endometriotic epithelial cells. *Mol Pharmacol* 2008; **73**:1394–1404.

Grunewald S, Jank A. New systemic agents in dermatology with respect to fertility, pregnancy, and lactation. *J Dtsch Dermatol Ges* 2015; **13**:277–289.

Guan P, Wang N. Mammalian target of rapamycin coordinates iron metabolism with iron-sulfur cluster assembly enzyme and tristetraprolin. *Nutrition* 2014; **30**:968–974.

Guo J, Gao J, Yu X, Luo H, Xiong X, Huang O. Expression of DJ-1 and mTOR in eutopic and ectopic endometria of patients with endometriosis and adenomyosis. *Gynecol Obstet Invest* 2015; **79**:195–200.

Halme J, Becker S, Hammond MG, Raj MH, Raj S. Increased activation of pelvic macrophages in infertile women with mild endometriosis. *Am J Obstet Gynecol* 1983; **145**:333–337.

Han Z, Boyle DL, Chang L, Bennett B, Karin M, Yang L, Manning AM, Firestein GS. c-Jun N-terminal kinase is required for metalloproteinase expression and joint destruction in inflammatory arthritis. *J Clin Invest* 2001; **108**:73–81.

Hay N, Sonnenberg N. Upstream and downstream of mTOR. *Genes Dev* 2004; **18**:1926–1945.

Hayakawa M, Miyashita H, Sakamoto I, Kitagawa M, Tanaka H, Yasuda H, Karin M, Kikugawa K. Evidence that reactive oxygen species do not mediate NF-kappaB activation. *EMBO J* 2003; **22**:3356–3366.

Hayden MS, West AP, Ghosh S. NF-kappaB and the immune response. *Oncogene* 2006; **25**:6758–6780.

Hennessy BT, Smith DL, Ram PT, Lu Y, Mills GB. Exploiting the PI3K/AKT pathway for cancer drug discovery. *Nat Rev Drug Discov* 2005; **4**:988–1004.

Hirata T, Osuga Y, Hamasaki K, Yoshino O, Ito M, Hasegawa A, Takemura Y, Hirota Y, Nose E, Morimoto C et al. Interleukin (IL)-17A stimulates IL-8 secretion, cyclooxygenase-2 expression, and cell proliferation of endometriotic stromal cells. *Endocrinology* 2008; **149**:1260–1267.

Hirata T, Osuga Y, Takamura M, Kodama A, Hirota Y, Koga K, Yoshino O, Harada M, Takemura Y, Yano T et al. Recruitment of CCR6-expressing Th17 cells by CCL20 secreted from IL-1 beta-, TNF-alpha-, and IL-17A-stimulated endometriotic stromal cells. *Endocrinology* 2010; **151**:5468–5476.

Hirota Y, Osuga Y, Hirata T, Harada M, Morimoto C, Yoshino O, Koga K, Yano T, Tsutsumi O, Taketani Y. Activation of protease-activated receptor 2 stimulates proliferation and interleukin (IL)-6 and IL-8 secretion of endometriotic stromal cells. *Hum Reprod* 2005; **20**:3547–3553.

Hornung D, Klingel K, Dohm K, Kandolf R, Wallwiener D, Taylor RN. Regulated on activation, normal T-cell-expressed and -secreted mRNA expression in normal endometrium and endometriotic implants. *Am J Pathol* 2001; **158**:1949–1954.

Huang F, Cao J, Liu Q, Zou Y, Li H, Yin T. MAPK/ERK signal pathway involved expression of COX-2 and VEGF by IL-1 $\beta$  induced in human endometriosis stromal cells in vitro. *Int J Clin Exp Pathol* 2013; **6**:2129–2136.

Hui L, Bakiri L, Mairhorfer A, Schweifer N, Haslinger C, Kenner L, Komnenovic V, Scheuch H, Beug H, Wagner EF. p38alpha suppresses normal and cancer cell proliferation by antagonizing the JNK-c-Jun pathway. *Nat Genet* 2007; **39**:741–749.

Hutson TE, Figlin RA, Kuhn JG, Motzer RJ. Targeted therapies for metastatic renal cell carcinoma: an overview of toxicity and dosing strategies. *The Oncologist* 2008; **13**:1084–1096.

Iizuka M, Igarashi M, Abe Y, Ibuki Y, Koyasu Y, Ikuma K. Chemical assay of iron in ovarian cysts: a new diagnostic method to evaluate endometriotic cysts. *Gynecol Obstet Invest* 1998; **46**:58–60.

Inoki K, Zhu T, Guan K-L. TSC2 mediates cellular energy response to control cell growth and survival. *Cell* 2003; **115**:577–590.

Jacobson TZ, Duffy JMN, Barlow D, Koninckx PR, Garry R. Laparoscopic surgery for pelvic pain associated with endometriosis. *Cochrane Database Syst Rev* 2009; **4**:CD001300.

Jana S, Paul S, Swarnkar S. Curcumin as anti-endometriotic agent: implication of MMP-3 and intrinsic apoptotic pathway. *Biochem Pharmacol* 2012; **83**:797–804.

Jäne PA, Gray N, Settleman J. Factors underlying sensitivity of cancers to small-molecule kinase inhibitors. *Nat Rev Drug Discov* 2009; **8**:709–723.

Javelaud D, Besançon F. NF- $\kappa$ B activation results in rapid inactivation of JNK in TNF alpha-treated Ewing sarcoma cells: a mechanism for the anti-apoptotic effect of NF- $\kappa$ B. *Oncogene* 2001; **20**:4365–4372.

Kaymakcalan MD, Je Y, Sonpavde G, Galsky M, Nguyen PL, Heng DYC, Richards CJ, Choueiri TK. Risk of infections in renal cell carcinoma (RCC) and non-RCC patients treated with mammalian target of rapamycin inhibitors. *Br J Cancer* 2013; **108**:2478–2484.

Khaled AR, Butifloski EJ, Sobel ES, Schiffenbauer J. Use of phosphorothioate-modified oligodeoxynucleotides to inhibit NF- $\kappa$ B expression and lymphocyte function. *Clin Immunol Immunopathol* 1998; **86**:170–179.

Khan N, Afaf F, Saleem M, Ahmad N, Mukhtar H. Targeting multiple signaling pathways by green tea polyphenol (-)-epigallocatechin-3-gallate. *Cancer Res* 2006; **66**:2500–2505.

Khan KN, Fujishita A, Kitajima M, Hiraki K, Nakashima M, Masuzaki H. Occult microscopic endometriosis: undetectable by laparoscopy in normal peritoneum. *Hum Reprod* 2014; **29**:462–472.

Kim K-H, Lee EN, Park JK, Lee J-R, Kim J-H, Choi H-J, Kim B-S, Lee H-W, Lee K-S, Yoon S. Curcumin attenuates TNF- $\alpha$ -induced expression of intercellular adhesion molecule-1, vascular cell adhesion molecule-1 and proinflammatory cytokines in human endometriotic stromal cells. *Phytother Res PTR* 2012; **26**:1037–1047.

Kim J-H, Jung S-H, Yang Y-I, Ahn J-H, Cho J-G, Lee K-T, Baek N-I, Choi J-H. Artemisia leaf extract induces apoptosis in human endometriotic cells through regulation of the p38 and NF $\kappa$ B pathways. *J Ethnopharmacol* 2013; **145**:767–775.

Kim TH, Yu Y, Luo L, Lydon JP, Jeong J-W, Kim JJ. Activated AKT pathway promotes establishment of endometriosis. *Endocrinology* 2014; **155**:1921–1930.

King AE, Critchley HO, Kelly RW. The NF- $\kappa$ B pathway in human endometrium and first trimester decidua. *Mol Hum Reprod* 2001; **7**:175–183.

King AE, Collins F, Klonisch T, Sallenave J-M, Critchley HOD, Saunders PTK. An additive interaction between the NF $\kappa$ B and estrogen receptor signalling pathways in human endometrial epithelial cells. *Hum Reprod* 2010; **25**:510–518.

Krajewska J, Handkiewicz-Junak D, Jarzab B. Sorafenib for the treatment of thyroid cancer: an updated review. *Expert Opin Pharmacother* 2015; **16**:573–583.

Kumar R, Clerc A-C, Gori I, Russell R, Pellegrini C, Govender L, Wyss J-C, Golshayan D, Canny GO. Lipoxin A<sub>2</sub> prevents the progression of de novo and established endometriosis in a mouse model by attenuating prostaglandin E<sub>2</sub> production and estrogen signaling. *PLoS One* 2014; **9**:e89742.

Kwok BH, Koh B, Ndubuisi MI, Eloffson M, Crews CM. The anti-inflammatory natural product parthenolide from the medicinal herb Feverfew directly binds to and inhibits Ik $\kappa$ B kinase. *Chem Biol* 2001; **8**:759–766.

Laird SM, Li TC, Bolton AE. The production of placental protein 14 and interleukin 6 by human endometrial cells in culture. *Hum Reprod* 1993; **8**:793–798.

Laudanski P, Szamatowicz J, Kowalcuk O, Kuzmicki M, Grabowicz M, Chyczewski L. Expression of selected tumor suppressor and oncogenes in endometrium of women with endometriosis. *Hum Reprod* 2009; **24**:1880–1890.

Lavon I, Goldberg I, Amit S, Landsman L, Jung S, Tsuberi BZ, Barshack I, Kopolovic J, Galun E, Bujard H et al. High susceptibility to bacterial infection, but no liver dysfunction, in mice compromised for hepatocyte NF- $\kappa$ B activation. *Nat Med* 2000; **6**:573–577.

Lebovic DI, Chao VA, Martini JF, Taylor RN. IL-1 $\beta$  induction of RANTES (regulated upon activation, normal T cell expressed and secreted) chemokine gene expression in endometriotic stromal cells depends on a nuclear factor- $\kappa$ B site in the proximal promoter. *J Clin Endocrinol Metab* 2001; **86**:4759–4764.

Lebovic DI, Kir M, Casey CL. Peroxisome proliferator-activated receptor-gamma induces regression of endometrial explants in a rat model of endometriosis. *Fertil Steril* 2004; **82**(Suppl 3):1008–1013.

Lebovic DI, Mwenda JM, Chai DC, Mueller MD, Santi A, Fisseha S, D'Hooghe T. PPAR-gamma receptor ligand induces regression of endometrial explants in baboons: a prospective, randomized, placebo- and drug-controlled study. *Fertil Steril* 2007; **88**:1108–1119.

Leconte M, Nicco C, Ngô C, Chéreau C, Chouzenoux S, Marut W, Guibourdenche J, Arkwright S, Weill B, Chapron C et al. The mTOR/AKT inhibitor temsirolimus prevents deep infiltrating endometriosis in mice. *Am J Pathol* 2011; **179**:880–889.

Leconte M, Santulli P, Chouzenoux S, Marcellin L, Cerles O, Chapron C, Dousset B, Batteux F. Inhibition of MAPK and VEGFR by Sorafenib controls the progression of endometriosis. *Reprod Sci* 2015; **22**:1171–1180.

Lee D-F, Hung M-C. All roads lead to mTOR: integrating inflammation and tumor angiogenesis. *Cell Cycle* 2007; **6**:3011–3014.

Lee DF, Kuo HP, Chen CT, Hsu JM, Chou CK, Wei Y, Sun HL, Li LY, Ping B, Huang WC et al. IKK beta suppression of TSC1 links inflammation and tumor angiogenesis via the mTOR pathway. *Cell* 2007; **130**:440–455.

Lee D-H, Kim S-C, Joo J-K, Kim H-G, Na Y-J, Kwak J-Y, Lee K-S. Effects of 17 $\beta$ -estradiol on the release of monocyte chemotactic protein-1 and MAPK activity in monocytes stimulated with peritoneal fluid from endometriosis patients. *J Obstet Gynaecol Res* 2012; **38**:516–525.

Leiro J, Arranz JA, Fraiz N, Sanmartín ML, Quezada E, Orallo F. Effect of cis-resveratrol on genes involved in nuclear factor kappa B signaling. *Int Immunopharmacol* 2005; **5**:393–406.

Lentsch AB, Shanley TP, Sarma V, Ward PA. In vivo suppression of NF- $\kappa$ B and preservation of I kappa B alpha by interleukin-10 and interleukin-13. *J Clin Invest* 1997; **100**:2443–2448.

Li Y, Inoki K, Vacratsis P, Guan K-L. The p38 and MK2 kinase cascade phosphorylates tuberin, the tuberous sclerosis 2 gene product, and enhances its interaction with 14-3-3. *J Biol Chem* 2003; **278**:13663–13671.

Li Y, Corradetti MN, Inoki K, Guan K-L. TSC2: Filling the GAP in the mTOR signaling pathway. *Trends Biochem Sci* 2004; **29**:32–38.

Li M-Q, Luo X-Z, Meng Y-H, Mei J, Zhu X-Y, Jin L-P, Li D-J. CXCL8 enhances proliferation and growth and reduces apoptosis in endometrial stromal cells in an autocrine manner via a CXCR1-triggered PTEN/AKT signal pathway. *Hum Reprod* 2012; **27**:2107–2116.

Li M-Q, Shao J, Meng Y-H, Mei J, Wang Y, Li H, Zhang L, Chang K-K, Wang X-Q, Zhu X-Y et al. NME1 suppression promotes growth, adhesion and implantation of endometrial stromal cells via Akt and MAPK/Erk1/2 signal pathways in the endometriotic milieu. *Hum Reprod* 2013; **28**:2822–2831.

Lin S-C, Wang C-C, Wu M-H, Yang S-H, Li Y-H, Tsai S-J. Hypoxia-induced microRNA-20a expression increases ERK phosphorylation and angiogenic gene expression in endometriotic stromal cells. *J Clin Endocrinol Metab* 2012; **97**:E1515–E1523.

Little AS, Smith PD, Cook SJ. Mechanisms of acquired resistance to ERK1/2 pathway inhibitors. *Oncogene* 2013; **32**:1207–1215.

Lousse J-C, Van Langendonck A, González-Ramos R, Defrère S, Renkin E, Donnez J. Increased activation of nuclear factor- $\kappa$ B (NF- $\kappa$ B) in isolated peritoneal macrophages of patients with endometriosis. *Fertil Steril* 2008; **90**:217–220.

Lousse J-C, Defrère S, Van Langendonck A, Gras J, González-Ramos R, Colette S, Donnez J. Iron storage is significantly increased in peritoneal macrophages of endometriosis patients and correlates with iron overload in peritoneal fluid. *Fertil Steril* 2009; **91**:1668–1675.

Ma XM, Blenner J. Molecular mechanisms of mTOR-mediated translational control. *Nat Rev Mol Cell Biol* 2009; **10**:307–318.

Maia H, Haddad C, Pinheiro N, Casoy J. Advantages of the association of resveratrol with oral contraceptives for management of endometriosis-related pain. *Int J Womens Health* 2012; **4**:543–549.

Maia H, Haddad C, Casoy J. Combining oral contraceptives with a natural nuclear factor- $\kappa$ B inhibitor for the treatment of endometriosis-related pain. *Int J Womens Health* 2013; **6**:35–39.

Maia H, Haddad C, Casoy J. The effect of pycnogenol on patients with dysmenorrhea using low-dose oral contraceptives. *Int J Womens Health* 2014; **6**:1019–1022.

Majumdar S, Lamothe B, Aggarwal BB. Thalidomide suppresses NF- $\kappa$ B activation induced by TNF and H2O<sub>2</sub>, but not that activated by ceramide, lipopolysaccharides, or phorbol ester. *J Immunol* 1990 2002; **168**:2644–2651.

Makinoshima H, Takita M, Saruwatari K, Umemura S, Obata Y, Ishii G, Matsumoto S, Sugiyama E, Ochiai A, Abe R et al. Signaling through the phosphatidylinositol 3-kinase (PI3K)/mammalian target of rapamycin (mTOR) axis is responsible for aerobic glycolysis mediated by glucose transporter in epidermal growth factor receptor (EGFR)-mutated lung adenocarcinoma. *J Biol Chem* 2015; **290**:17495–17504.

Maleka A, Enblad G, Sjörs G, Lindqvist A, Ullenhag GJ. Treatment of metastatic malignant melanoma with vemurafenib during pregnancy. *J Clin Oncol* 2013; **31**:e192–e193.

Manning BD. Balancing Akt with S6K: implications for both metabolic diseases and tumorigenesis. *J Cell Biol* 2004; **167**:399–403.

Matsuzaki S, Darcha C. Co-operation between the AKT and ERK signaling pathways may support growth of deep endometriosis in a fibrotic microenvironment in vitro. *Hum Reprod* 2015; **30**:1606–1616.

McKinnon B, Bersinger NA, Huber AW, Kuhn A, Mueller MD. PPAR-gamma expression in peritoneal endometriotic lesions correlates with pain experienced by patients. *Fertil Steril* 2010; **93**:293–296.

McKinnon B, Bersinger NA, Mueller MD. Peroxisome proliferating activating receptor gamma-independent attenuation of interleukin 6 and interleukin 8 secretion from primary endometrial stromal cells by thiazolidinediones. *Fertil Steril* 2012a; **97**:657–664.

McKinnon B, Bersinger NA, Wotzkow C, Mueller MD. Endometriosis-associated nerve fibers, peritoneal fluid cytokine concentrations, and pain in endometriotic lesions from different locations. *Fertil Steril* 2012b; **97**:373–380.

McKinnon BD, Evers J, Bersinger NA, Mueller MD. Induction of the neurokinin 1 receptor by TNF $\alpha$  in endometriotic tissue provides the potential for neurogenic control over endometriotic lesion growth. *J Clin Endocrinol Metab* 2013; **98**:2469–2477.

McKinnon B, Bertschi D, Wotzkow C, Bersinger NA, Evers J, Mueller MD. Glucose transporter expression in eutopic endometrial tissue and ectopic endometriotic lesions. *J Mol Endocrinol* 2014; **52**:169–179.

McKinnon BD, Bertschi D, Bersinger NA, Mueller MD. Inflammation and nerve fiber interaction in endometriotic pain. *Trends Endocrinol Metab* 2015; **26**:1–10.

Mei J, Li M-Q, Ding D, Li D-J, Jin L-P, Hu W-G, Zhu X-Y. Indoleamine 2,3-dioxygenase-1 (IDO1) enhances survival and invasiveness of endometrial stromal cells via the activation of JNK signaling pathway. *Int J Clin Exp Pathol* 2013; **6**:431–444.

Menzies AM, Yeh I, Botton T, Bastian BC, Scolyer RA, Long GV. Clinical activity of the MEK inhibitor trametinib in metastatic melanoma containing BRAF kinase fusion. *Pigment Cell Melanoma Res* 2015; **28**:607–610.

Meuleman C, Vandenabeele B, Fieuws S, Spiessens C, Timmerman D, D'Hooghe T. High prevalence of endometriosis in infertile women with normal ovulation and normospermic partners. *Fertil Steril* 2009; **92**:68–74.

Meuleman C, Tomassetti C, D'Hoore A, Van Cleynenbreugel B, Penninckx F, Vergote I, D'Hooghe T. Surgical treatment of deeply infiltrating endometriosis with colorectal involvement. *Hum Reprod Update* 2011; **17**:311–326.

Miller MA, Meyer AS, Beste MT, Lasisi Z, Reddy S, Jeng KW, Chen C-H, Han J, Isaacson K, Griffith LG et al. ADAM-10 and -17 regulate endometriotic cell migration via concerted ligand and receptor shedding feedback on kinase signaling. *Proc Natl Acad Sci* 2013; **110**:E2074–E2083.

Montenegro MA, Palomino H. Induction of cleft palate in mice by inhibitors of prostaglandin synthesis. *J Craniofac Genet Dev Biol* 1990; **10**:83–94.

Moravek MB, Ward EA, Lebovic DI. Thiazolidinediones as therapy for endometriosis: a case series. *Gynecol Obstet Invest* 2009; **68**:167–170.

Morotti M, Vincent K, Brawn J, Zondervan KT, Becker CM. Peripheral changes in endometriosis-associated pain. *Hum Reprod Update* 2014; **20**:717–736.

Morris EJ, Jha S, Restaino CR, Dayananth P, Zhu H, Cooper A, Carr D, Deng Y, Jin W, Black S et al. Discovery of a novel ERK inhibitor with activity in models of acquired resistance to BRAF and MEK inhibitors. *Cancer Discov* 2013; **3**:742–750.

Murphy AA, Palinski W, Rankin S, Morales AJ, Parthasarathy S. Evidence for oxidatively modified lipid-protein complexes in endometrium and endometriosis. *Fertil Steril* 1998; **69**:1092–1094.

Nasu K, Nishida M, Ueda T, Yuge A, Takai N, Narahara H. Application of the nuclear factor- $\kappa$ B inhibitor BAY 11-7085 for the treatment of endometriosis: an in vitro study. *Am J Physiol Endocrinol Metab* 2007; **293**:E16–E23.

Ngô C, Chéreau C, Nicco C, Weill B, Chapron C, Batteux F. Reactive oxygen species controls endometriosis progression. *Am J Pathol* 2009; **175**:225–234.

Ngô C, Nicco C, Leconte M, Chéreau C, Arkwright S, Vacher-Lavenu M-C, Weill B, Chapron C, Batteux F. Protein kinase inhibitors can control the progression of endometriosis in vitro and in vivo. *J Pathol* 2010; **222**:148–157.

Nidai Ozes O, Mayo LD, Gustin JA, Pfeffer SR, Pfeffer LM, Donner DB. NF- $\kappa$ B activation by tumour necrosis factor requires the Akt serine–threonine kinase. *Nature* 1999; **401**:82–85.

Nirgianakis K, McKinnon B, Imboden S, Knabben L, Gloor B, Mueller MD. Laparoscopic management of bowel endometriosis: resection margins as a predictor of recurrence. *Acta Obstet Gynecol Scand* 2014; **93**:1262–1267.

Nisolle M, Donnez J. Peritoneal endometriosis, ovarian endometriosis, and adenomyotic nodules of the rectovaginal septum are three different entities. *Fertil Steril* 1997; **68**:585–596.

Noble LS, Simpson ER, Johns A, Bulun SE. Aromatase expression in endometriosis. *J Clin Endocrinol Metab* 1996; **81**:174–179.

OEckinghaus A, Hayden MS, Ghosh S. Crosstalk in NF- $\kappa$ B signaling pathways. *Nat Immunol* 2011; **12**:695–708.

Ohama Y, Harada T, Iwabe T, Taniguchi F, Takenaka Y, Terakawa N. Peroxisome proliferator-activated receptor-gamma ligand reduced tumor necrosis factor-alpha-induced interleukin-8 production and growth in endometriotic stromal cells. *Fertil Steril* 2008; **89**:311–317.

Ohori M, Takeuchi M, Maruki R, Nakajima H, Miyake H. FR180204, a novel and selective inhibitor of extracellular signal-regulated kinase, ameliorates collagen-induced arthritis in mice. *Naunyn Schmiedebergs Arch Pharmacol* 2007; **374**:311–316.

Olivares C, Bilotas M, Buquet R, Borghi M, Suello C, Tesone M, Meresman G. Effects of a selective cyclooxygenase-2 inhibitor on endometrial epithelial cells from patients with endometriosis. *Hum Reprod* 2008; **23**:2701–2708.

Olivares C, Ricci A, Bilotas M, Barañoa RI, Meresman G. The inhibitory effect of celecoxib and rosiglitazone on experimental endometriosis. *Fertil Steril* 2011; **96**:428–433.

Olivares CN, Bilotas MA, Ricci AG, Barañoa RI, Meresman GF. Anastrozole and celecoxib for endometriosis treatment, good to keep them apart? *Reprod* 2013; **145**:119–126.

Oner G, Ozcelik B, Ozgun MT, Serin IS, Ozturk F, Basbug M. The effects of metformin and letrozole on endometriosis and comparison of the two treatment agents in a rat model. *Hum Reprod* 2010; **25**:932–937.

Oner-Yıldırım Y, Koçak H, Gürdöll F, Korkmaz D, Buyru F. Indices of oxidative stress in eutopic and ectopic endometria of women with endometriosis. *Gynecol Obstet Invest* 2004; **57**:214–217.

OuYang Z, Hirota Y, Osuga Y, Hamasaki K, Hasegawa A, Tajima T, Hirata T, Koga K, Yoshino O, Harada M et al. Interleukin-4 stimulates proliferation of endometriotic stromal cells. *Am J Pathol* 2008; **173**:463–469.

Ozcan Cenksoy P, Oktem M, Erdem O, Karakaya C, Cenksoy C, Erdem A, Guner H, Karabacak O. A potential novel treatment strategy: inhibition of angiogenesis and inflammation by resveratrol for regression of endometriosis in an experimental rat model. *Gynecol Endocrinol* 2015; **31**:219–224.

Park SB, Jee BC, Kim SH, Cho YJ, Han M. Cyclooxygenase-2 inhibitor, celecoxib, inhibits leiomyoma cell proliferation through the nuclear factor  $\kappa$ B pathway. *Reprod Sci* 2014; **21**:1187–1195.

Pause A, Méthot N, Svitkin Y, Merrick WC, Sonenberg N. Dominant negative mutants of mammalian translation initiation factor eIF-4A define a critical role for eIF-4F in cap-dependent and cap-independent initiation of translation. *EMBO J* 1994; **13**:1205–1215.

Peng Q, Wei Z, Lau BH. Pycnogenol inhibits tumor necrosis factor-alpha-induced nuclear factor kappa B activation and adhesion molecule expression in human vascular endothelial cells. *Cell Mol Life Sci* 2000; **57**:834–841.

Pierce JW, Schoenleber R, Jesmok G, Best J, Moore SA, Collins T, Gerritsen ME. Novel Inhibitors of cytokine-induced  $\text{I}\kappa\text{B}\alpha$  phosphorylation and endothelial cell adhesion molecule expression show anti-inflammatory effects in vivo. *J Biol Chem* 1997; **272**:21096–21103.

Rahmioglu N, Nyholt DR, Morris AP, Missmer SA, Montgomery GW, Zondervan KT. Genetic variants underlying risk of endometriosis: insights from meta-analysis of eight genome-wide association and replication datasets. *Hum Reprod* 2014; **29**:702–716.

Rashmi R, DeSelms C, Helms C, Bowcock A, Rogers BE, Rader J, Grigsby PW, Schwarz JK. AKT inhibitors promote cell death in cervical cancer through disruption of mTOR signaling and glucose uptake. *PLoS One* 2014; **9**:e92948.

Remorgida V, Ragni N, Ferrero S, Anserini P, Torelli P, Fulcheri E. How complete is full thickness disc resection of bowel endometriotic lesions? A prospective surgical and histological study. *Hum Reprod* 2005; **20**:2317–2320.

Ricci AG, Olivares CN, Bilotas MA, Bastón JL, Singla JJ, Meresman GF, Barañoa RI. Natural therapies assessment for the treatment of endometriosis. *Hum Reprod* 2013; **28**:178–188.

Rodriguez-Viciano P, Warne PH, Dhand R, Vanhaesebrouck B, Gout I, Fry MJ, Waterfield MD, Downward J. Phosphatidylinositol-3-OH kinase as a direct target of Ras. *Nature* 1994; **370**:527–532.

Roux PP, Ballif BA, Anjum R, Gygi SP, Blenis J. Tumor-promoting phorbol esters and activated Ras inactivate the tuberous sclerosis tumor suppressor complex via p90 ribosomal S6 kinase. *Proc Natl Acad Sci* 2004; **101**:13489–13494.

Rudzitis-Auth J, Mengen MD, Laschke MW. Resveratrol is a potent inhibitor of vascularization and cell proliferation in experimental endometriosis. *Hum Reprod* 2013; **28**:1339–1347.

Sakamoto Y, Harada T, Horie S, Iba Y, Taniguchi F, Yoshida S, Iwabe T, Terakawa N. Tumor necrosis factor-alpha-induced interleukin-8 (IL-8) expression in endometriotic stromal cells, probably through nuclear factor-kappa B activation: gonadotropin-releasing hormone agonist treatment reduced IL-8 expression. *J Clin Endocrinol Metab* 2003; **88**:730–735.

Sampson JA. Peritoneal Endometriosis due to the menstrual dissemination of endometrial tissue into the peritoneal cavity. *Am J Obstet Gynecol* 1927; **14**:422–469.

Sanfilippo JS, Williams RS, Yussman MA, Cook CL, Bissonnette F. Substance P in peritoneal fluid. *Am J Obstet Gynecol* 1992; **166**:155–159.

Santulli P, Borghese B, Chouzenoux S, Vaiman D, Borderie D, Streuli I, Goffinet F, De Ziegler D, Weill B, Batteux F et al. Serum and peritoneal interleukin-33 levels are elevated in deeply infiltrating endometriosis. *Hum Reprod* 2012; **27**:2001–2009.

Santulli P, Borghese B, Chouzenoux S, Streuli I, Borderie D, de Ziegler D, Weill B, Chapron C, Batteux F. Interleukin-19 and interleukin-22 serum levels are decreased in patients with ovarian endometrioma. *Fertil Steril* 2013; **99**:219–226.

Santulli P, Chouzenoux S, Fiorese M, Marcellin L, Lemarechal H, Millischer AE, Batteux F, Borderie D, Chapron C. Protein oxidative stress markers in peritoneal fluids of women with deep infiltrating endometriosis are increased. *Hum Reprod* 2015a; **30**:49–60.

Santulli P, Marcellin L, Tosti C, Chouzenoux S, Cerles O, Borghese B, Batteux F, Chapron C. MAP kinases and the inflammatory signaling cascade as targets for the treatment of endometriosis? *Expert Opin Ther Targets* 2015b; **19**:1–19.

Sato N, Tsunoda H, Nishida M, Morishita Y, Takimoto Y, Kubo T, Noguchi M. Loss of heterozygosity on 10q23.3 and mutation of the tumor suppressor gene PTEN in benign endometrial cyst of the ovary: possible sequence progression from benign endometrial cyst to endometrioid carcinoma and clear cell carcinoma of the ovary. *Cancer Res* 2000; **60**:7052–7056.

Sawyers CL. Opportunities and challenges in the development of kinase inhibitor therapy for cancer. *Genes Dev* 2003; **17**:2998–3010.

Seo SK, Yang HI, Lee KE, Kim HY, Cho S, Choi YS, Lee BS. The roles of thioredoxin and thioredoxin-binding protein-2 in endometriosis. *Hum Reprod* 2010; **25**:1251–1258.

Serhan CN, Chiang N, Van Dyke TE. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. *Nat Rev Immunol* 2008; **8**:349–361.

Seval Y, Cakmak H, Kayisli UA, Arici A. Estrogen-mediated regulation of p38 mitogen-activated protein kinase in human endometrium. *J Clin Endocrinol Metab* 2006; **91**:2349–2357.

Shaw RW. Treatment of endometriosis. *Lancet* 1992; **340**:1267–1271.

Shen F, Wang Y, Lu Y, Yuan L, Liu X, Guo S-W. Immunoreactivity of progesterone receptor isoform B and nuclear factor kappa-B as biomarkers for recurrence of ovarian endometriomas. *Am J Obstet Gynecol* 2008; **199**:486.e1–e486.e10.

Shimizu A, O'Brien KP, Sjöblom T, Pietras K, Buchdunger E, Collins VP, Heldin C-H, Dumanski JP, Östman A. The Dermatofibrosarcoma protuberans-associated collagen type Iα1/platelet-derived growth factor (PDGF) B-chain fusion gene generates a transforming protein that is processed to functional PDGF-BB. *Cancer Res* 1999; **59**:3719–3723.

Simoens S, Dunselman G, Dirksen C, Hummelshøj L, Bokor A, Brandes I, Brodszky V, Canis M, Colombo GL, DeLeire T et al. The burden of endometriosis: costs and quality of life of women with endometriosis and treated in referral centres. *Hum Reprod* 2012; **27**:1292–1299.

Simon M-P, Pedeutour F, Sirvent N, Grosgeorge J, Minoletti F, Coindre J-M, Terrier-Lacombe M-J, Mandahl N, Craver R, Blin N et al. Dereulation of the platelet-derived growth factor β-chain gene via fusion with collagen gene COL1A1 in dermatofibrosarcoma protuberans and giant-cell fibroblastoma. *Nat Genet* 1997; **15**:95–98.

Sizemore N, Leung S, Stark GR. Activation of phosphatidylinositol 3-kinase in response to interleukin-1 leads to phosphorylation and activation of the NF-κB p65/RelA subunit. *Mol Cell Biol* 1999; **19**:4798–4805.

Smale ST. Hierarchies of NF-κB target-gene regulation. *Nat Immunol* 2011; **12**:689–694.

Somigliana E, Infantino M, Candiani M, Vignali M, Chiodini A, Busacca M, Vignali M. Association rate between deep peritoneal endometriosis and other forms of the disease: pathogenetic implications. *Hum Reprod* 2004; **19**:168–171.

Somigliana E, Vercellini P, Gattei U, Chopin N, Chiodo I, Chapron C. Bladder endometriosis: getting closer and closer to the unifying metastatic hypothesis. *Fertil Steril* 2007; **87**:1287–1290.

Straus DS, Pascual G, Li M, Welch JS, Ricote M, Hsiang CH, Sengchanthalangsy LL, Ghosh G, Glass CK. 15-deoxy-delta 12,14-prostaglandin J2 inhibits multiple steps in the NF-κB signaling pathway. *Proc Natl Acad Sci* 2000; **97**:4844–4849.

Streuli I, de Ziegler D, Santulli P, Marcellin L, Borghese B, Batteux F, Chapron C. An update on the pharmacological management of endometriosis. *Expert Opin Pharmacother* 2013; **14**:291–305.

Stumvoll M, Nurjhan N, Perriello G, Dailey G, Gerich JE. Metabolic effects of metformin in non-insulin-dependent diabetes mellitus. *N Engl J Med* 1995; **333**:550–554.

Su F, Viros A, Milagre C, Trunzer K, Bollag G, Spleiss O, Reis-Filho JS, Kong X, Koya RC, Flaherty KT et al. RAS mutations in cutaneous squamous-cell carcinomas in patients treated with BRAF inhibitors. *N Engl J Med* 2012; **366**:207–215.

Sun HS, Hsiao K-Y, Hsu C-C, Wu M-H, Tsai S-J. Transactivation of steroidogenic acute regulatory protein in human endometriotic stromal cells is mediated by the prostaglandin EP2 receptor. *Endocrinology* 2003; **144**:3934–3942.

Tagashira Y, Taniguchi F, Harada T, Ikeda A, Watanabe A, Terakawa N. Interleukin-10 attenuates TNF-alpha-induced interleukin-6 production in endometriotic stromal cells. *Fertil Steril* 2009; **91**:2185–2192.

Takai E, Taniguchi F, Nakamura K, Uegaki T, Iwabe T, Harada T. Parthenolide reduces cell proliferation and prostaglandin E2 [corrected] in human endometriotic stromal cells and inhibits development of endometriosis in the murine model. *Fertil Steril* 2013; **100**:1170–1178.

Tang F, Tang G, Xiang J, Dai Q, Rosner MR, Lin A. The Absence of NF-κB-mediated inhibition of c-Jun N-terminal kinase activation contributes to tumor necrosis factor alpha-induced apoptosis. *Mol Cell Biol* 2002; **22**:8571–8579.

Taniguchi F, Harada T, Miyakoda H, Iwabe T, Deura I, Tagashira Y, Miyamoto A, Watanabe A, Suou K, Uegaki T et al. TAK1 activation for cytokine synthesis and proliferation of endometriotic cells. *Mol Cell Endocrinol* 2009; **307**:196–204.

Taniguchi F, Higaki H, Azuma Y, Deura I, Iwabe T, Harada T, Terakawa N. Gonadotropin-releasing hormone analogues reduce the proliferation of endometrial stromal cells but not endometriotic cells. *Gynecol Obstet Invest* 2013; **75**:9–15.

Teague EMCO, Print CG, Hull ML. The role of microRNAs in endometriosis and associated reproductive conditions. *Hum Reprod Update* 2010; **16**:142–165.

Tilton F, La Du JK, Vue M, Alzarban N, Tanguay RL. Dithiocarbamates have a common toxic effect on zebrafish body axis formation. *Toxicol Appl Pharmacol* 2006; **216**:55–68.

Tong C, Yin Z, Song Z, Dockendorff A, Huang C, Mariadason J, Flavell RA, Davis RJ, Augenlicht LH, Yang W. c-Jun NH2-terminal kinase I plays a critical role in intestinal homeostasis and tumor suppression. *Am J Pathol* 2007; **171**:297–303.

Trukhacheva E, Lin Z, Reierstad S, Cheng Y-H, Milad M, Bulun SE. Estrogen receptor (ER) beta regulates ERα expression in stromal cells derived from ovarian endometriosis. *J Clin Endocrinol Metab* 2009; **94**:615–622.

Urata Y, Osuga Y, Izumi G, Takamura M, Koga K, Nagai M, Harada M, Hirata T, Hirota Y, Yoshino O et al. Interleukin-1β stimulates the secretion of thymic stromal lymphopoietin (TSLP) from endometrioma stromal cells: possible involvement of TSLP in endometriosis. *Hum Reprod* 2012; **27**:3028–3035.

Veillat V, Lavoie CH, Metz CN, Roger T, Labelle Y, Akoum A. Involvement of nuclear factor-κB in macrophage migration inhibitory factor gene transcription up-regulation induced by interleukin-1 beta in ectopic endometrial cells. *Fertil Steril* 2009; **91**:2148–2156.

Veillat V, Carli C, Metz CN, Al-Abed Y, Naccache PH, Akoum A. Macrophage migration inhibitory factor elicits an angiogenic phenotype in human ectopic endometrial cells and triggers the production of major angiogenic factors via CD44, CD74, and MAPK signaling pathways. *J Clin Endocrinol Metab* 2010; **95**:E403–E412.

Velarde MC, Aghajanova L, Nezhat CR, Giudice LC. Increased mitogen-activated protein kinase kinase/extracellularly regulated kinase activity in human endometrial stromal fibroblasts of women with endometriosis reduces 3',5'-cyclic adenosine 5'-monophosphate inhibition of cyclin D1. *Endocrinology* 2009; **150**:4701–4712.

Venturelli S, Berger A, Böcker A, Busch C, Weiland T, Noor S, Leischner C, Schleicher S, Mayer M, Weiss TS et al. Resveratrol as a pan-HDAC inhibitor alters the acetylation status of histone [corrected] proteins in human-derived hepatoblastoma cells. *PLoS One* 2013; **8**:e73097.

Vercellini P, Aimi G, Panazza S, Vicentini S, Pisacreta A, Crosignani PG. Deep endometriosis conundrum: evidence in favor of a peritoneal origin. *Fertil Steril* 2000; **73**:1043–1046.

Vercellini P, Somigliana E, Viganò P, Abbiati A, Daguati R, Crosignani PG. Endometriosis: current and future medical therapies. *Best Pract Res Clin Obstet Gynaecol* 2008; **22**:275–306.

Vercellini P, Crosignani PG, Abbiati A, Somigliana E, Viganò P, Fedele L. The effect of surgery for symptomatic endometriosis: the other side of the story. *Hum Reprod Update* 2009; **15**:177–188.

Vercellini P, Viganò P, Somigliana E, Fedele L. Endometriosis: pathogenesis and treatment. *Nat Rev Endocrinol* 2014; **10**:261–275.

Wang Y, Chen H, Wang N, Guo H, Fu Y, Xue S, Ai A, Lyu Q, Kuang Y. Combined 17 $\beta$ -estradiol with TCDD promotes M2 polarization of macrophages in the endometriotic milieu with aid of the interaction between endometrial stromal cells and macrophages. *PLoS One* 2015; **10**:e0125559.

Weston CR, Davis RJ. The JNK signal transduction pathway. *Curr Opin Genet Dev* 2002; **12**:14–21.

Wieser F, Vigne J-L, Ryan I, Hornung D, Djalali S, Taylor RN. Sulindac suppresses nuclear factor- $\kappa$ B activation and RANTES gene and protein expression in endometrial stromal cells from women with endometriosis. *J Clin Endocrinol Metab* 2005; **90**:6441–6447.

Wilhelm SM, Carter C, Tang L, Wilkie D, McNabola A, Rong H, Chen C, Zhang X, Vincent P, McHugh M et al. BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. *Cancer Res* 2004; **64**:7099–7109.

Wu M-H, Wang C-A, Lin C-C, Chen L-C, Chang W-C, Tsai S-J. Distinct regulation of cyclooxygenase-2 by interleukin-1 $\beta$  in normal and endometriotic stromal cells. *J Clin Endocrinol Metab* 2005; **90**:286–295.

Wu M-H, Lin S-C, Hsiao K-Y, Tsai S-J. Hypoxia-inhibited dual-specificity phosphatase-2 expression in endometriotic cells regulates cyclooxygenase-2 expression. *J Pathol* 2011; **225**:390–400.

Wu R, Zhou W, Chen S, Shi Y, Su L, Zhu M, Chen Q, Chen Q. Lipoxin A4 suppresses the development of endometriosis in an ALX receptor-dependent manner via the p38 MAPK pathway. *Br J Pharmacol* 2014; **171**:4927–4940.

Wullschleger MF, Imboden S, Wanner J, Mueller MD. Minimally invasive surgery when treating endometriosis has a positive effect on health and on quality of work life of affected women. *Hum Reprod* 2015; **30**:553–557.

Xiu-li W, Su-ping H, Hui-hua D, Zhi-xue Y, Shi-long F, Pin-hong L. NF- $\kappa$ B decoy oligonucleotides suppress RANTES expression and monocyte chemotactic activity via NF- $\kappa$ B inactivation in stromal cells of ectopic endometrium. *J Clin Immunol* 2009; **29**:387–395.

Xu JJ, Hendriks BS, Zhao J, de Graaf D. Multiple effects of acetaminophen and p38 inhibitors: towards pathway toxicology. *FEBS Lett* 2008; **582**:1276–1282.

Xu H, Becker CM, Lui WT, Chu CY, Davis TN, Kung AL, Birsner AE, D'Amato RJ, Wai Man GC, Wang CC. Green tea epigallocatechin-3-gallate inhibits angiogenesis and suppresses vascular endothelial growth factor C/vascular endothelial growth factor receptor 2 expression and signaling in experimental endometriosis in vivo. *Fertil Steril* 2011; **96**:1021–1028.

Xue Q, Lin Z, Cheng Y-H, Huang C-C, Marsh E, Yin P, Milad MP, Confino E, Reierstad S, Innes J et al. Promoter methylation regulates estrogen receptor 2 in human endometrium and endometriosis. *Biol Reprod* 2007; **77**:681–687.

Yagyu T, Kobayashi H, Matsuzaki H, Wakahara K, Kondo T, Kurita N, Sekino H, Inagaki K, Suzuki M, Kanayama N et al. Thalidomide inhibits tumor necrosis factor- $\alpha$ -induced interleukin-8 expression in endometriotic stromal cells, possibly through suppression of nuclear factor- $\kappa$ B activation. *J Clin Endocrinol Metab* 2005; **90**:3017–3021.

Yagyu T, Tsuji Y, Haruta S, Kitanaka T, Yamada Y, Kawaguchi R, Kanayama S, Tanase Y, Kurita N, Kobayashi H. Activation of mammalian target of rapamycin in postmenopausal ovarian endometriosis. *Int J Gynecol Cancer* 2006; **16**:1545–1551.

Yamaguchi K, Mandai M, Toyokuni S, Hamanishi J, Higuchi T, Takakura K, Fujii S. Contents of endometriotic cysts, especially the high concentration of free iron, are a possible cause of carcinogenesis in the cysts through the iron-induced persistent oxidative stress. *Clin Cancer Res* 2008; **14**:32–40.

Yamamoto Y, Gaynor RB. Therapeutic potential of inhibition of the NF- $\kappa$ B pathway in the treatment of inflammation and cancer. *J Clin Invest* 2001; **107**:135–142.

Yamauchi N, Harada T, Taniguchi F, Yoshida S, Iwabe T, Terakawa N. Tumor necrosis factor- $\alpha$  induced the release of interleukin-6 from endometriotic stromal cells by the nuclear factor- $\kappa$ B and mitogen-activated protein kinase pathways. *Fertil Steril* 2004; **82**(Suppl 3):1023–1028.

Yilmaz B, Sucak A, Kilic S, Aksakal O, Aksoy Y, Lortlar N, Sut N, Gungor T. Metformin regresses endometriotic implants in rats by improving implant levels of superoxide dismutase, vascular endothelial growth factor, tissue inhibitor of metalloproteinase-2, and matrix metalloproteinase-9. *Am J Obstet Gynecol* 2010; **202**:e1–e8.

Yin X, Pavone ME, Lu Z, Wei J, Kim JJ. Increased activation of the PI3K/AKT pathway compromises decidualization of stromal cells from endometriosis. *J Clin Endocrinol Metab* 2012; **97**:E35–E43.

Yoon S, Seger R. The extracellular signal-regulated kinase: multiple substrates regulate diverse cellular functions. *Growth Factors* 2006; **24**:21–44.

Yoshino O, Osuga Y, Hirota Y, Koga K, Hirata T, Harada M, Morimoto C, Yano T, Nishii O, Tsutsumi O et al. Possible pathophysiological roles of mitogen-activated protein kinases (MAPKs) in endometriosis. *Am J Reprod Immunol* 2004; **52**:306–311.

Yoshino O, Osuga Y, Koga K, Hirota Y, Hirata T, Rui Meng X, Na L, Yano T, Tsutsumi O, Taketani Y, FR 167653, a p38 mitogen-activated protein kinase inhibitor, suppresses the development of endometriosis in a murine model. *J Reprod Immunol* 2006; **72**:85–93.

Yotova IY, Quan P, Leditzniq N, Beer U, Wenzl R, Tschugguel W. Abnormal activation of Ras/Raf/MAPK and RhoA/ROCKII signalling pathways in eutopic endometrial stromal cells of patients with endometriosis. *Hum Reprod* 2011; **26**:885–897.

Yu L, McPhee CK, Zheng L, Mardones GA, Rong Y, Peng J, Mi N, Zhao Y, Liu Z, Wan F et al. Termination of autophagy and reformation of lysosomes regulated by mTOR. *Nature* 2010; **465**:942–946.

Zakikhani M, Dowling R, Fantus IG, Sonenberg N, Pollak M. Metformin is an AMP kinase-dependent growth inhibitor for breast cancer cells. *Cancer Res* 2006; **66**:10269–10273.

Zarubin T, Han J. Activation and signaling of the p38 MAP kinase pathway. *Cell Res* 2005; **15**:11–18.

Zeitoun K, Takayama K, Sasano H, Suzuki T, Moghrabi N, Andersson S, Johns A, Meng L, Putman M, Carr B et al. Deficient 17 $\beta$ -hydroxysteroid dehydrogenase type 2 expression in endometriosis: failure to metabolize 17 $\beta$ -estradiol. *J Clin Endocrinol Metab* 1998; **83**:4474–4480.

Zhang J, Yang PL, Gray NS. Targeting cancer with small molecule kinase inhibitors. *Nat Rev Cancer* 2009; **9**:28–39.

Zhang H, Zhao X, Liu S, Li J, Wen Z, Li M. 17 $\beta$ E2 promotes cell proliferation in endometriosis by decreasing PTEN via NF- $\kappa$ B-dependent pathway. *Mol Cell Endocrinol* 2010a; **317**:31–43.

Zhang J, Xu Z, Dai H, Ji X, Duan Y, Zhang C, Qin D. Application of the nuclear factor- $\kappa$ B inhibitor pyrrolidine dithiocarbamate for the treatment of endometriosis: an in vitro study. *Fertil Steril* 2010b; **94**:2942–2944.

Zhang J, Xu Z, Chang H, Zhang C, Dai H, Ji X, Li C, Wang X. Pyrrolidine dithiocarbamate attenuates nuclear factor- $\kappa$ B activation, cyclooxygenase-2 expression and prostaglandin E2 production in human endometriotic epithelial cells. *Gynecol Obstet Invest* 2011; **72**:163–168.

Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, Wu M, Ventre J, Doepper T, Fujii N et al. Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest* 2001; **108**:1167–1174.

Zhou W-D, Yang H-M, Wang Q, Su D-Y, Liu F-A, Zhao M, Chen Q-H, Chen Q-X. SB203580, a p38 mitogen-activated protein kinase inhibitor, suppresses the development of endometriosis by down-regulating proinflammatory cytokines and proteolytic factors in a mouse model. *Hum Reprod* 2010; **25**:3110–3116.